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# Proposal – 009459

**Submitted**

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# [COPY] Longitudinal Imaging of Cortical Bone Remodeling in a Rabbit Osteoporosis Model: A Novel Platform for the Study of Bone Physiology, Disease and Treatment

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## Abstract

Cortical bone tissue continuously renews itself throughout life via remodeling, which is dictated by specialized groups of bone cells known as Basic Multicellular Units (BMUs) through coupled bone resorption and formation. However, with increasing age bone resorption outweighs formation, which can lead to degenerative diseases such as osteoporosis (OP). Current knowledge of the factors that affect BMU activity is largely based on fluorochrome labelling of bone microarchitecture and 2D histomorphometry which limits our understanding of the mechanisms that affect the formative activities of BMUs within their natural 3D context. This knowledge gap has significant consequences for our understanding of bone health and disease - we do not know how disease states that contribute to OP impact this balance of resorption and formation. By using a comparable rabbit remodeling model to human remodeling, we will characterize cortical bone microarchitectural changes via increasing Activation Frequency (AF) - the number of BMUs present within a given bone - to which parathyroid hormone 1-34 (human) (PTH) treatment is a proven model. Increasing AF rates, and thus the ability to observe BMU remodeling on a large scale, will significantly increase our understanding of the mechanisms responsible for both the regulation and subsequent breakdown of bone.

## Scientific Description

Bone tissue continuously renews itself throughout life via remodeling, which is dictated by specialized groups of bone cells known as Basic Multicellular Units (BMUs). In cortical bone, BMUs dictate remodeling through coupled bone resorption and formation (Fig. 1). However, with increasing age this process becomes unbalanced, bone resorption outweighs formation which can lead to degenerative diseases such as osteoporosis (OP). Globally, 200 million people live with osteoporosis (OP) (www.iofbonehealth.org). This disease costs Canada $4.6 billion each year due to its high prevalence and

chronic nature (Hopkins et al., 2016). Fractures related to OP are more common than heart attack, stroke and breast cancer combined (www.osteoporosis.ca). With 1 in 3 women and 1 in 5 men expected to experience an OP-related fracture in their lifetimes (www.osteoporosis.ca), no Canadian family will escape the human and economic impacts. Our current understanding of the factors that affect BMU activity is largely based on fluorochrome labelling of bone microarchitecture and 2D histomorphometry which limits our understanding of the mechanisms that affect the formative activities of BMUs within their natural three-dimensional (3D) context. This knowledge gap has significant consequences for our understanding of bone health and disease - we do not know how disease states that contribute to OP impact this balance of resorption and formation. Thus, strategies for the prevention and/or reversal of OP ultimately must seek to augment this balance. The need for balance and not simply cessation of remodeling is a point driven home by the rapidly growing body of literature focused on the negative side-effects (osteonecrosis of the jaw, atypical fractures) of some anti-resorptive therapies for OP - mainly bisphosphonates, but also recently reported for Denosumab (Yoneda et al., 2017; Fung et al., 2016; Khan et al., 2016; Khow et al., 2017; Adler, RA, 2016). Given their role in bone adaptation and disease, the ability to track remodeling events in cortical bone by detection of their porous resorption spaces has the potential to be transformative for our understanding of bone physiology and disease. Currently, little is known about the three-dimensional (3D) spatio-temporal behavior of remodeling events in cortical bone and much of the inquiry in this area is theoretical and/or computational in nature (Buenzli et al., 2011; 2014; Pivonka et al., 2008; van Oers et al., 2008; Ryser et al., 2009; Martin, RB, 2007; Smit TH and EH Burger, 2000; Smit et al., 2002). Direct empirical four-dimensional (4D; 3D over time) data have been beyond reach for several reasons including the small scale of cortical pores in preclinical models, the high radiation dose necessary to detect them and the fact that the most common preclinical models of OP (mice and rats) do not exhibit cortical bone remodeling. We seek to overcome these limitations through synchrotron-based X-ray phase contrast imaging of a rabbit model of OP. The American Food and Drug Administration recommends the use of a larger animal model which remodels its cortex in addition to the common ovariectomy (OVX) rat model for OP studies (Thompson et al., 1995) and thus rabbits serve as a good model to investigate BMU remodeling behavior. That said, for this model to be operationally effective, there is the need to increase Activation Frequency (AF) which is the number of BMUs present within a given bone to which OVX plus glucocorticoid (GC) and human parathyroid hormone (PTH) treatments are proven models. In fall 2017, our group carried out a pilot study to identify which OP model (OVX+GC and PTH) would prove successful for increasing AF for subsequent in vivo tracking of BMU remodeling in the cortical bone of the rabbit (n=35). During cycle 27 our group will be imaging, in vivo, OVX+GC treated rabbits, however due to the overwhelming success of both models (Fig. 2), we are submitting a proposal in cycle 28 to image PTH treated rabbits. Radiation dose effect on AF rates will also be investigated. Rabbits (n=15/cycle; 30 total) will be imaged at 2, 5 and 10 Gy thus, the two OP models will be divided into consecutive cycles 27 (OXV+GC rabbits) and 28 (PTH rabbits) for logistical purposes. The goal of this proposal is to: 1. Characterize cortical bone microarchitectural changes in the rabbit through the implementation of the PTH model and 2. Optimize 4D synchrotron-based imaging of individual remodeling events in this model system. This study will set the stage for the next phase of this program of research which will strive to evaluate current and novel pharmaceuticals as well as new treatment regimens on the remodeling of cortical bone, at a level that has not been possible before which can prove invaluable for the fight against bone degenerative diseases like osteoporosis.

# Capability & Productivity of Research Team

a) Approximately three shifts of our beamteam time in cycle 21 was used to test imaging the ex vivo rabbit tibia. We developed a scanning protocol using phase-contrast in-line micro-CT that yielded the necessary resolution while imparting the targeted radiation dose. The cortical canal network of the rabbit was successfully visualized with this optimized scanning protocol which gave us the confidence to pursue live rabbit imaging at the end of cycle 23. b) In cycle 24, we executed the first ever live rabbit imaging at BMIT using a low (~1.0 Gy; n = 6) ultra-low (~0.5 Gy; n = 6) protocol at baseline, followed by a 2 week observation period for any effects of radiation, and then another scan. We successfully rendered the cortical bone tissue in the tibia (Fig. 3) and tracked remodeling at the level of the BMU for the first time (Fig. 4). Future work from this data will be carried out to confirm that the radiation from the imaging has not effected the rate of BMU progression by using a series of bone-labeling fluorochrome injections (Baumann et al., 2015). c) In cycle 25, we tested Glucocorticoid (GC) as an Osteoporosis model for the purpose of increasing AF and thus the number of remodeling BMUs in three fluorochrome labeled rabbits from cycle 24 animals. Results did show signs of remodeling, however this was only seen extensively in one of the three animals treated and thus, the next step was to identify the method best suited for increasing AF rates in the cortical bone of New Zealand white rabbits for subsequent in vivo imaging at the CLS.

In Fall 2017, we carried out a large pilot study consisting of 35 rabbits to test OP models (OVX, GC, OVX+GC, PTH and sham). Ex vivo, Micro-CT scans (1172 Bruker) have indicated both OVX+GC and PTH increased remodeling (Fig. 2). During cycle 27 our group will be imaging, in vivo OVX+GC treated rabbits, however due to the overwhelming success of both models (Fig. 2), we are requesting beamtime in cycle 28 to image PTH treated rabbits. Radiation dose effect on AF rates will also be investigated. Rabbits (n=15/cycle; 30 total) will be imaged at 2, 5 and 10 Gy thus, the two OP models will be divided into consecutive cycles 27 and 28 for logistical purposes. Inducing remodeling will ultimately provide us the means to observe and track the behavior of BMU activity, in vivo, in live animals which will significantly enhance our current understanding of bone biology and the factors that lead to degenerative diseases such as osteoporosis.

Past Productivity

Dr. David Cooper is an Associate Professor and Tier II Canada Research Chair in Synchrotron Bone Imaging at the University of Saskatchewan. His group has extensively employed BMIT in experimental projects focused on 3D imaging of bone and other materials. Related outputs are listed below. Trainees of Dr. Cooper are marked with double asterisks (**).

Summary: 28 papers Published and 3 supervised theses since 2010

CLS Related Productivity (25 published/Accepted) Accepted/ In Press /Published Manuscripts:

Accepted. Pratt **et al. Interpreting the three dimensional orientation of vascular canals and cross-sectional geometry of cortical bone in birds and bats. Journal of Anatomy. 2017. Bukejs et al. Contributions to the palaeofauna of Ptinidae (Coleptera) known from Baltic amber. Zootaxa 4344 (1), 181-188. 2017. Simovich et al. Superhydrophobicity from the Inside. Langmuir. 2017. Andronowski** et al. Occurrence of osteon banding in adult human cortical bone. American Journal of Physical Anthropology. 164(3): 635-642. 2017 Andronowski **et al. Evaluating differential nuclear DNA yield rates and osteocyte numbers among human bone tissue types: A synchrotron radiation micro-CT approach. Forensic Science International: Genetics 28, 211-218 2017. Pratt** and Cooper. A method for measuring the three-dimensional orientation of cortical canals with implications for comparative analysis of bone microstructure in vertebrates. Micron 92, 32-38 2016. Melli et al. A sparsity-based iterative algorithm for reconstruction of micro-CT images from highly undersampled projection datasets obtained with a synchrotron X-ray source. Review of Scientific Instruments 87 (12), 123701 2016 Panahifar A**, et al. Spectral K-edge Subtraction Imaging of Experimental Non-Radioactive Barium Uptake in Bone. Physica Medica: European Journal of Medical Physics. 32 (12), 1765-1770. 2016 Panahifar A**, et al. Three-dimensional labeling of newly formed bone using synchrotron radiation barium K-edge subtraction imaging. Med. Phys. Biol. 61 (13), 5077. 2015. Wiebe, S. et al. Biomedical Imaging Using Synchrotron Radiation: Experience at the Biomedical Imaging and Therapy (BMIT) Facility at the Canadian Light Source. Synch. Rad. News. 2015 28(5):16-23. 2015. Harrison KD **and Cooper DML. Three dimensional visualization of cortical bone remodeling: the past, present and near future. Front. Endocrinol., 11 August 2015. 2015. Karunakaran C, et al. Factors influencing real time internal structural visualization and dynamic process monitoring in plants using synchrotron-based phase contrast X-ray imaging. Sci Rep. Jul 17;5:12119. 2015. Panahifar A**, et al. 3-D localization of non-radioactive strontium in osteoarthritic bone: role in the dynamic labeling of bone pathological changes. J. Orth Res. Accepted (April 27. 2015). 2015. Rhoades G, et al. Diffraction Enhanced-Computed Tomography Imaging of Growing Joints Using a Synchrotron Light Source: A Model for Studying Juvenile-Onset Arthritis. Comp. Med. 2015 Apr 18;65(4):342-7. 2015. Pratt I.V**, et al. In vivo imaging of rat cortical bone porosity by synchrotron phase contrast micro computed tomography. Phys Med in Biol. Jan 7;60(1):211-32

Completed Theses focused on Synchrotron Data (including that from BMIT): 2014 Carter Y.**. In Vivo Imaging of Cortical Porosity by Synchrotron Phase Contrast Micro Computed Tomography. Ph.D. Thesis. Department of Anatomy and Cell Biology, U. of Saskatchewan. 2013 Pratt, I.V.**. In Vivo Imaging of Cortical Porosity by Synchrotron Phase Contrast Micro Computed Tomography. M.Sc. Thesis. Department of Anatomy and Cell Biology, U. of Saskatchewan. 2013 Chisti, A.**. Texture Analysis of Diffraction Enhanced Synchrotron Images of Trabecular Bone at the Wrist. M.Sc. Thesis. Department of Computer Science, U. of Saskatchewan. (Co-supervised by Drs. Cooper and Eramian).

# Societal, Economic and Industrial Relevance

SOCIETAL AND ECONOMIC IMPACT: N/A INDUSTRIAL RELEVANCE: N/A

# Materials & Methods

## **BMIT-ID** – Biomedical Imaging and Therapy ID Beamline

**15 Shifts**

### Suitability and Justification:

The synchrotron radiation phase-contrast micro-CT available at the CLS overcomes both the limitations of image quality and radiation dose compared to available desktop micro-CT when used to track the progression of cortical bone remodeling in an animal model such as the rabbit. Based on previous work by the Cooper lab at the BMIT-ID beamline, it is clear that the CLS is unique for live animal imaging and has both the resolution to image cortical remodeling and the high flux monochromatic beam combined with phase-contrast imaging to deliver a significantly reduced radiation dose. In addition to the fact that the current BMIT-ID stage setup is uniquely optimized to hold a live rabbit in the path of the beam successfully for imaging, the current setup is also unprecedentedly rapid. On the ID line the duration of the ex vivo tibia scan was only ~30 seconds, in contrast to previous in vivo scanning protocols used by our lab for rats on the bending magnet line which were in excess of 10 minutes. The rapidity of the scanning protocol is immeasurably beneficial to in vivo scanning as the rabbit is anesthetized for a much shorter time and thus much less likely to move during the scan.

| | |
|---|---|
| **Source** | Superconducting Wiggler |
| **Spectral Range** | 25 - 150 keV |
| **Resolution** | CT, KES:10^-3 DEI:10^-5 |
| **Spot Sizes** | 220 mm x 11 mm @ 55 m |
| **Photon Flux** | 3 x $10^{12}$ ph /(s * $mr^2$ * 0.1%bW * mA) @ 20 keV |

### Biomedical Imaging and Therapy ID Beamline (BMIT-ID)

The BioMedical Imaging and Therapy (BMIT) Facility is designed for the purpose of imaging biological tissue and conducting radiation therapy research. The BMIT facility will address the interest of scientists and clinicians in the diagnosis and treatment of cancer, circulatory and respiratory disease, neurological and behavioural disease, reproductive dysfunction, musculo-skeletal disease and kinesiology, and dental conditions.
http://bmit.lightsource.ca

### Experimental Procedure:

**In-line (Propagation-Based) Phase Contrast Imaging (PBI)** **Conventional Absorption Imaging**
**Computed Tomography (voxel size >= 2um^3) (CT)** **Micro Computed Tomography (voxel size < 1um^3) (uCT)**

Skeletally mature, 6-month old female New Zealand White rabbits will be divided into 3 groups of 5 animals each: 2 Gy, 5 Gy and 10 Gy. Animals will receive PTH injections of 30 ug/kg/day for 4 weeks. All animals will undergo injection with the bone-labelling fluorochrome, calcein to facilitate dynamic histomorphometry. Calcein will be administered after two weeks (days 13 and 14) and four weeks (days 27 and 28). Phase contrast micro-CT imaging of the right distal tibia will be performed in vivo 2 weeks after PTH injections have started (2 weeks prior to euthanasia) at the BioMedical Imaging and Therapy (BMIT) facility at the CLS. The rabbits will be restrained in a custom holder adapted from a design by Voor et al., (2008) and modified from a prototype utilized in our preliminary studies. Rabbits will be anesthetized according to an established protocol using a transnasal delivery of a sedative and anesthetic cocktail (Dexmedetomidine, Midazolam, Butorphanol) and inhaled isofluorane (Santangelo et al., 2016). Imaging will be performed at 12.8 m voxel size (Hamamatsu Orca Flash 4.0 camera system with a AA40 beam monitoring system) and 60 cm target to detector distance at 40 keV as established in proof-of-principle experiments (Figs. 2 and 3). The field of view will be approximately 1 cm high and 3 cm wide which readily encompasses the width of the distal tibia. Animals will be imaged at 2, 5 and 10 Gy doses. We have employed 2-3 Gy extensively in rats with no apparent radiation effects (~1-3 Gy being commonly used in laboratory micro-CT systems (Pratt et al., 2015)), 5 Gy is the highest dose previously reported in the literature for in vivo (trabecular) bone imaging at a synchrotron (Matsumoto et al., 2011) and we anticipate effects should be evident at 10 Gy which enters the therapeutic realm but given the short timeframe (2 weeks) potential impacts are unclear. Following imaging, the rabbits will be injected with anesthetic reversal drugs (Flumazenil and Atipamezole) and recovered. Animals will be monitored daily for an additional two weeks after which they will be euthanized and imaged ex vivo at BMIT using the same configuration, but at the high dose of 10 Gy for all groups to maximize image quality. Anatomical landmarks based upon the ankle joint will ensure accurate targeting of the same region of interest. Contralateral tibiae will also be scanned at this point as a negative control for AF and overall BMU morphology. Assuming a 40 um/day Longitudinal Rate of Erosion,

BMUs should advance 560 um between scans - a scale that is readily detectable by the 13 m voxel size (Fig. 4). Dr. Cooper, as a member and leader of the BMIT beam team, has guaranteed beam time which will ensure the proposed research can proceed without delay. All team members will be involved in animal handling/monitoring, imaging and data analysis. Animal welfare veterinarians Dr. Melanie Gibbons and/or Dr. Kurtis Swekla will oversee anesthetic and monitoring procedures. Based on our previous experience with imaging live rabbits, we are requesting the maximum number of 15 shifts.

### Ancillary Requirements:

**Labs:** BMIT Lab

**Equipment:** Anesthesia equipment

# Materials

## Samples:

28G09459~Harrison

## Electrical Equipment:

| Name | Description | Manufacturer | Model |
|---|---|---|---|
| PhysioSuite | Animal monitoring system | Kent Scientific | PS0746 (PhysioSuite) |

## Sample Preparation:

CHEMICAL MATERIALS SAMPLES

Chemical Materials(CM) Involved: Y Oxygen cylinders are located inside the hutch and are connected to the anesthesia cart. Isoflurane is located in a glass bottle and connected to the anesthesia cart via the cart's anesthesia key. Anesthesia drugs used for trans-nasal delivery and reversal are kept secured in a locked cabinet located in the Large Animal Lab. Post beamtime, drugs are not kept on the premises.

LIVE ANIMAL SAMPLES

Live Animal(LA) Involved: Y a) Animals will be transported to and from the CLS in the LASU vehicle by LASU staff. b) Rabbits will be anesthetized according to an established protocol using a transnasal delivery of a sedative and anesthetic cocktail (Dexmedetomidine, Midazolam, Butorphanol) and inhaled isofluorane. The sedative will be administered in the Large Animal lab. Once sedated, animals will be carried into the hutch and placed on isoflurane via the anesthesia cart and monitored via our PhysioSuite. Once, stable, animals will then be placed in a custom holder on the stage. During imaging, vitals are taken every 5 minutes and animals are monitored outside the hutch via in-hutch cameras. After scanning, animals are administered reversal drugs (Flumazenil and Atipamezole) and placed on oxygen. Upon waking, animals are placed inside recovery cages inside the Large Animal lab and further monitored and supported with saline fluids.

NANOMATERIALS

NM-How are the samples contained a substrate:
N/A

NON HAZARDOUS MATERIALS SAMPLES

Non-Hazardous Material(NHM) Involved: Y Saline solution is kept inside the Large Animal Lab and does not require any specific handling/storage procedures.

ANIMAL TISSUE SAMPLES

Animal Tissue(AT) Involved: Y Post-euthanasia, dissected, frozen lower hindlimbs (both left and right) of the rabbits will be imaged ex vivo. When not being imaged, the hindlimbs will be kept in the -20 freezer in the Large Animal Lab. Once beamtime is complete, hindlimbs will be removed from the freezer and taken out of the CLS.

## Waste Generation:

The following types of waste will be generated:

- Biological syringes, blades, glass etc.

## Waste Disposal:

AT-Solid Waste Disposal Procedure: Syringes/needles used for injecting anesthetic/reversal drugs will be disposed of in the appropriate sharps containers.

# Appendix: Attachments

| File | Type | Owner | Size | Added |
|---|---|---|---|---|
| Images/Refs | Scientific | Kim Harrison | 340.4 KB | 2018-03-07 13:15 |
| Ethics approval | Ethics | Kim Harrison | 50.9 KB | 2018-03-07 13:17 |