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Spectral K-edge subtraction imaging of experimental non-radioactive barium uptake in bone

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ABSTRACT

Purpose: To evaluate the feasibility of using non-radioactive barium as a bone tracer for detection with synchrotron spectral K-edge subtraction (SKES) technique.
Methods: Male rats of 1-month old (*i.e.,* developing skeleton) and 8-month old (*i.e.,* skeletally mature) were orally dosed with low dose of barium chloride (33 mg/kg/day Ba^{2+}) for 4 weeks. The fore and hind limbs were dissected for imaging in projection and computed tomography modes at 100 μm and 52 μm pixel sizes. The SKES method utilizes a single bent Laue monochromator to prepare a 550 eV energy spectrum to encompass the K-edge of barium (37.441 keV), for collecting both 'above' and 'below' the K-edge data sets in a single scan.
Results: The SKES has a very good focal size, thus limits the 'crossover' and motion artifacts. In juvenile rats, barium was mostly incorporated in the areas of high bone turnover such as at the growth plate and the trabecular surfaces, but also in the cortical bone as the animals were growing at the time of tracer administration. However, the adults incorporated approximately half the concentration and mainly in the areas where bone remodeling was predominant and occasionally in the periosteal and endosteal layers of the diaphyseal cortical bone.
Conclusions: The presented methodology is simple to implement and provides both structural and functional information, after labeling with barium, on bone micro-architecture and thus has great potential for *in vivo* imaging of pre-clinical animal models of musculoskeletal diseases to better understand their mechanisms and to evaluate the efficacy of pharmaceuticals.

1. Introduction

Molecular imaging is usually associated with nuclear medicine in clinical settings and with the addition of florescence and infrared based imaging in basic sciences research. However, molecular imaging can also be performed using absorption-based X-ray imaging, if a suitable contrast agent (*i.e.,* tracer) is available. Dual energy K-edge subtraction imaging (KES) utilizes the discontinuity in the attenuation coefficient of an element at its K-edge to specifically detect distribution of that element. This can be achieved using synchrotron sources [1–4] and with X-ray tubes [5,6]. The focus of this article is on synchrotron techniques as the more sensitive and developed of the approaches. In KES imaging, two data sets are collected from the sample; one at slightly above and one at slightly below the K-edge of the element of interest, followed by their subtraction. Because the energy difference of these two data sets is typically on the order of several hundreds of eV, the absorption properties of other elements are only negligibly different, therefore, the outcome is the specific map of the tracer. Depending on the nature of experiment, different data collection strategies can be used. Temporal subtraction of the X-ray energies provides more sensitive data, but is only suitable for *ex vivo* and *in vitro* experiments due to the need for two separate data acquisitions [1,2]. Whereas in simultaneous subtraction methods a narrow range of X-ray energies encompasses the K-edge, thus both data sets are collected at the same time, making this approach more suitable for *in vivo* experiments [3,7]. Typically, the two X-ray energies are prepared by using a focusing bent Laue crystal (as opposed to double Bragg crystals in the temporal method) and placing a splitter in the centre of the beam as they converge towards the sample; the splitter separates the X-ray beams into energies above and below the K-edge. However, this approach

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inherently suffers from a geometrical error due to the interaction of the two beams with the sample at a crossover angle. This is of a less concern if the sample to be imaged is homogenous or composed of materials with similar absorption properties, but becomes problematic at the interfaces of two distinct materials such as bone and soft tissue.

The physiological process of bone turnover (*i.e.,* remodeling) lies at the root of many diseases such as osteoporosis, osteoarthritis, bone cancers, *etc.* In order to better understand the role of remodeling in bone diseases, we aimed to combine anatomical imaging of bone with functional imaging of new bone formation/turnover at high resolution, after dynamic labeling with non-radioactive barium in rats. Barium, similar to calcium, when bioavailable will incorporate into the hydroxyapatite mineral of newly forming bone, and is normally only present in minute concentrations in the body, thus when administered experimentally it can be used as a tracer of bone formation. To visualize spatial localization of this barium tracer in bone we used a recently modified version of the simultaneous subtraction method called spectral KES (SKES) [4]. This approach utilizes a bent Laue monochromator, but without the splitter, that leads to a faster and more sensitive scans, while also minimizing the 'crossover' artifact. This is possible because of the better energy dispersion of the monochromator which reduces the spectral energy blurring of previous systems. The reduced energy blurring allows the SKES system to use beam closer to the K-edge (less crossover angle) and offers higher flux (no blocked beam due to the splitter). The SKES method has previously been successfully tested on phantoms and *ex vivo* imaging of lungs of a mouse injected with iodine [4]. In the current study we evaluated simultaneous KES imaging of barium incorporation in skeleton which is more challenging due to the distinctively different attenuation coefficients of bone and soft tissues. This initial proof-of-principle study focused on *ex vivo* imaging of long bones, with soft tissues attached, to acquire projection and computed tomography (CT) data, but given the promising outcomes, SKES appears as a viable method for imaging bone remodeling in live pre-clinical animal models of musculoskeletal disease.

2. Materials and methods

2.1. Animals

Barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$, ⩾99.9% purity, MW 244.26 g/mol) was purchased from Sigma-Aldrich (Oakville, Ontario). Four groups of healthy Sprague-Dawley male rats were included in the study: 1) Young rats of 1 month-old age (n = 5) (*i.e.,* developing skeleton); 2) Adults of 8 month-old age (n = 5) (*i.e.,* skeletally mature); 3) Young controls (n = 3); and 4) Adult controls (n = 3). Barium chloride was dissolved in distilled water and dosed to animals orally with a curved feeding needle at the dosage of 58.5 mg/kg/day (equivalent to 33 mg/kg/day free Ba^{2+}) for 28 days. Animals were fasted 2 h before and after the administration to minimize competitive uptake of Ca by bone during the tracer administration. After euthanization, fore and hind limbs were dissected and frozen for later SKES imaging. The animal use protocol (# 20110124) was approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. Spectral K-edge subtraction imaging

Barium distribution in the bone was evaluated in projection (2D) and CT (3D) mode by SKES technique at the BioMedical Imaging and Therapy (BMIT) bending magnet beamline [8] at the Canadian Light Source (CLS). The schematic set up of the imaging system is presented in Fig. 1. The bent Laue monochromator used in this system provides a narrow spectral range of about 550 eV enabling imaging both 'above' and 'below' energies around the K-edge of barium (37.441 keV) simultaneously (Fig. 2). A (3,1,1) reflection from a Si (5,1,1) wafer was selected [4]. The size of the beam at focus was about 90 μm.

Barium phantoms were prepared through serial dilution of barium chloride in distilled water. Although lower concentrations seemed to be detectable, we did not test concentrations lower than 690 μg/mL (Fig. 3). Bone samples were scanned at 100 μm pixel size using a flat panel detector. Moreover, projections and CT

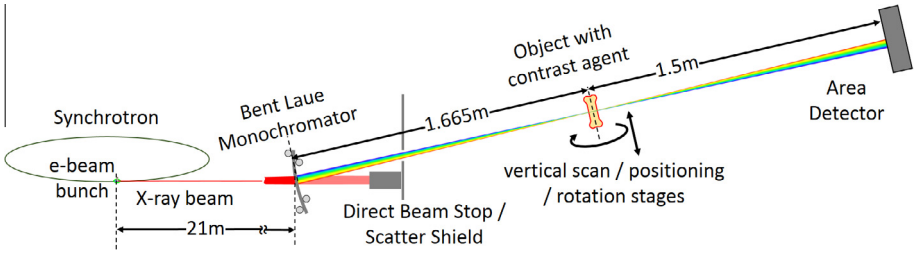

Fig. 1. SKES imaging system. The image shows a schematic design of the SKES imaging system. Note that the distance between sample and detector is slightly less than the distance between crystal and sample due to the shortage of the rail that holds the system. The rail system was initially optimized for imaging iodine, but can be easily modified. In addition, if the space in experimental hutch allows, increasing the focal length will minimize the crossover artifact by the same factor.

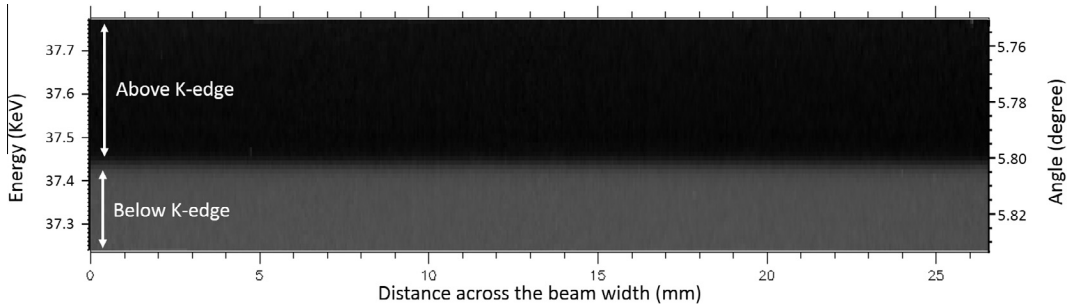

Fig. 2. Beam profile. The image shows the beam profile when the barium tank was placed in the beam path. The energy range in the SKES setup was about 550 eV. The image is flat and dark field corrected and was captured with a Hamamatsu ORCA-Flash 4.0 detector (Field of view: ~90 μm * 26 mm, Barium K-edge: 37.44 keV).

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