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Differential accumulation of lead and zinc in double-tidemarks of articular cartilage

A. Roschger †‡*, J.G. Hofstaetter †§‖, B. Pemmer ‡, N. Zoeger ‡¶, P. Wobrauschek ‡, G. Falkenberg #, R. Simon ††, A. Berzlanovich ‡‡, H.W. Thaler §§, P. Roschger †, K. Klaushofer †, C. Streli ‡

† *Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria*
‡ *Atominstitut, Technische Universitaet Wien, Stationallee 2, 1020 Vienna, Austria*
§ *Department of Orthopedics, Vienna General Hospital, Medical University of Vienna, 1090 Vienna, Austria*
‖ *2nd Department, Orthopaedic Hospital Vienna-Speising, Vienna, Austria*
¶ *Nuclear Engineering Seibersdorf GmbH, 2444 Seibersdorf, Austria*
# *Hamburger Synchrotronstrahlungslabor HASYLAB am Deutschen Elektronen-Synchrotron, Notkestr. 85, 22603 Hamburg, Germany*
†† *Karlsruhe Institute of Technology, Institute for Synchrotron Radiation, Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany*
‡‡ *Department of Forensic Medicine, Medical University of Vienna, A-1090 Vienna, Austria*
§§ *AUVA Trauma Centre Meidling, Austria*

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SUMMARY

Introduction: Long-term exposure to increased lead (Pb) concentrations is associated with several chronic diseases. The divalent cation zinc (Zn) is essential for numerous enzymes. In a recent study we found remarkably elevated concentrations of Pb and Zn in the tidemark (TM), which is the mineralization front of human articular cartilage.
Objective: Duplication or multiplication of TMs occurs with advancing age or degeneration. We hypothesized that trace elements accumulate in TMs as a function of time. Thus, in cases of double TMs, the deep (older) TM should contain higher Pb and Zn concentrations than the superficial (younger) TM.
Design: Undecalcified tissue from articular cartilage and subchondral bone of femoral heads and patellae was examined by synchrotron radiation induced confocal micro X-ray fluorescence analysis and by quantitative backscattered electron imaging to determine the local distribution of Ca, Zn, and Pb in this tissue.
Results: The evaluation of X-ray fluorescence intensities in double TMs revealed in average a 2.6-fold higher Pb level in the deep TM compared to the superficial TM while Zn concentrations were similar. Pb and Zn contents were significantly enhanced in the deep TM (Pb: 35-fold, Zn: five-fold) and in the superficial TM (Pb: 12-fold, Zn: five-fold) compared to the bone level.
Conclusion: For the first time a differential accumulation of Pb and Zn is documented in regions with double TMs revealing various timescales for the accumulation of these elements. Increased amounts of Pb are present in the TMs (up to the 62-fold of the bone level) featuring a potential source of internal Pb release if the TM region is destroyed.

Introduction

The element lead (Pb) is a toxic heavy metal and the exposure to Pb is associated with chronic diseases at the nervous, hematopoietic, skeletal, renal, and endocrine system[1]. It is known that Pb accumulates in the skeleton, where approximately 95% of the total body burden is present[2]. Osteoblasts and chondrocytes seem to be important target cells for the toxic effects of Pb[3]. Clinical studies indicate that exposure to Pb can lead to osteopenia[4,5], osteoporosis[6], impaired bone healing[7], and even osteoarthritis[8–10]. Furthermore, it

* Address correspondence and reprint requests to: A. Roschger, Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Kundratstrasse 37, A-1120 Vienna, Austria. Tel: 43-1601502650; Fax: 43-1601502651.
E-mail addresses: andreas.roschger@osteologie.at (A. Roschger), jochen.hofstaetter@oss.at (J.G. Hofstaetter), bpemmer@ati.ac.at (B. Pemmer), norbert.zoeger@nes.at (N. Zoeger), wobi@ati.ac.at (P. Wobrauschek), gerald.falkenberg@desy.de (G. Falkenberg), r.simon@kit.edu (R. Simon), andrea.berzlanovich@meduniwien.ac.at (A. Berzlanovich), Heinrich.Thaler@auva.at (H.W. Thaler), paul.roschger@osteologie.at (P. Roschger), Klaus.Klaushofer@osteologie.at (K. Klaushofer), streli@ati.ac.at (C. Streli).

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was shown that whole blood Pb levels are associated with biomarkers of joint tissue metabolism and may play a role in osteoarthritis[11]. Nelson *et al.* found a correlation between elevated blood Pb levels and the incidence of osteoarthritis but question remained if this is the result of a direct toxic effect of Pb on the joint tissue or if Pb is released from the mineralized tissue[12]. In contrast to Pb, zinc (Zn) is an essential trace element, widespread in the reactive center of various enzymes and crucial for normal growth of the skeleton[13]. Even though the role of Zn in bone metabolism is still unknown, recent studies assumed stimulating effects on bone formation *via* facilitation of osteoblastic cell proliferation[14]. On the other hand a study on Zn deficient rats indicated no change in bone mineral density, turnover, architecture, or biomechanics relative to controls[15]. Until recently, the spatial distribution of Pb and Zn elements in cartilage and bone were unknown. Using confocal synchrotron radiation induced micro X-ray fluorescence (SR-μXRF) analysis, we could characterize the spatial distribution of Pb and Zn in the osteochondral region of normal adult human joints[16]. Interestingly, we found a highly specific accumulation of Pb and Zn at the interphase between mineralized and non-mineralized articular cartilage (non-mdAC), the so-called tidemark (TM)[17].

The TM is of clinical importance since clefts may open in the cartilage due to injuries and osteoarthritis[18,19]. The TM represents the mineralization front in articular cartilage and can be stained with tetracycline labeling[20,21]. Moreover, double or even multiple TMs are observed, when periods of extra-phase mineralization occur. The number of TMs in the femoral head is reported to increase with age. While below 60 years the average number of TMs varies between 1 and 1.5 older individuals (above 70) have in average 1.5–2.5 TMs[22]. A three-dimensional study of patella samples revealed a significantly increased TM area in osteoarthritis (7.7 cm^2) compared to controls (2.6 cm^2)[22,23]. Whenever a new mineralization front starts to advance, the older TM is entrapped in newly mdAC matrix. Therefore, the spatial sequence of TMs also reflects a temporal sequence. However, little is known about mechanisms and dynamics of the mineralization process of cartilage as well as of the accumulation of Pb and Zn, as observed in single TMs previously[16].

In the present work, our hypothesis was that Pb and Zn are accumulated with time when the mineralization front had stopped to advance. Hence, the deep TM (deepTM) should have in average always a higher concentration of Pb and Zn than the superficial TM (supTM). To prove this assumption we investigated osteochondral samples from patella and femoral head displaying double TMs using confocal SR-μXRF in combination with backscattered electron imaging (BEI).

Material and methods

The study was approved by the ethics committee of the Medical University of Vienna, Austria (EK 638/2007) and was done in accordance with the Helsinki Declaration.

Bone samples with double TMs

Osteochondral samples from patellas were obtained from forensic autopsies (#1, #2). Samples from femoral heads were retrieved from forensic autopsies (sample #3, #5) or following hip arthroplasty after osteoporotic femoral neck fractures (sample #4, #6, #7, #8) (Table I). About 5 mm thick undecalcified blocks of the Polymethylmethacrylat (PMMA) embedded samples were cut perpendicular to the articular surface from the central region of the femoral head (frontal plane) and of patella (sagittal plane). Sample preparation prior to quantitative backscattered electron imaging (qBEI) was performed as described elsewhere[24–26]. The analyzed samples were part of a sample set of 40 specimens, which were screened for double TMs after qBEI analysis. The osteochondral samples containing double TMs were further investigated by SR-μXRF [$n = 6$ femoral heads and $n = 2$ patellae (Table I)]. All patients had no history of work-related Pb exposure. The average age of the individuals was 64 years (ranging from 33 to 85). Additionally one femoral head sample (#9, Table I) with end-stage osteoarthritis was analyzed, which had completely worn-out cartilage. This sample was excluded from the main study and is discussed separately.

Table I
List of analyzed samples (n is the number of corresponding measurement fields)

Sample (n)	Tissue	Sex	Age (years)	Origin	Synchrotron beam line
1 (2)	Patella	M	46	Autopsy	Hasylab, beam L
2 (5)	Patella	M	54	Autopsy	Hasylab, beam L
3 (1)	Femoral head	F	65	Autopsy	Hasylab, beam L
4 (3)	Femoral head	F	72	Osteoporotic/hip replacement	Hasylab, beam L
5 (3)	Femoral head	F	33	Autopsy	ANKA, FLUO
6 (1)	Femoral head	F	76	Osteoporotic/hip replacement	ANKA, FLUO
7 (1)	Femoral head	F	85	Osteoporotic/hip replacement	ANKA, FLUO
8 (1)	Femoral head	F	79	Osteoporotic/hip replacement	ANKA, FLUO
9 (1)	Femoral head	M	62	Osteoarthritic/hip replacement	Hasylab, beam L

qBEI

qBEI is a validated technique to visualize and to quantify the calcium concentration distribution in bone and mineralized cartilage with a spatial resolution <1 μm. Therefore, areas with bright gray levels reflect well-mineralized matrix with high Ca content, whereas areas with dark gray levels indicate low mineral density.

A digital scanning electron microscope (DSM 962, Zeiss, Oberkochen, Germany) equipped with a four-quadrant semiconductor BE-detector was employed to obtain the qBEI data. The microscope was operated at an acceleration voltage of 20 kV with a working distance set to 15 mm. The probe current was maintained at 110 pA. More details on this method can be found elsewhere[24–26]. Images at 200× magnification (1 μm spatial resolution) were used for data evaluation.

SR-μXRF – analysis

SR-μXRF is a powerful analytical tool for qualitative and semi-quantitative analysis of chemical elements, based on the detection of characteristic X-rays induced by primary high-energy photons. The sensitivity reaches the femtogram range for medium Z elements. Details on confocal SR-μXRF can be found elsewhere[16,27–32].

The measurements of this study were performed at confocal SR-μXRF setups at the micro focus end-station of HASYLAB beam line L (Hamburg, Germany)[33,34] and at the ANKA FLUO beam line (Karlsruhe, Germany)[31,35]. Two matched polycarpellary half lenses from XOS (X-Ray Optical Systems, New York, USA) were installed in the incident beam and in front of the detector enabling a well-defined observation volume just below the sample surface.

At HASYLAB beam line L a Ni/C fixed exit double multilayer monochromator, providing a high photon flux, was used to set the excitation energy of the primary beam to 18 keV. The size of the detection volume of 20 μm × 14 μm × 22 μm (horizontal × vertical depth) at 9.7 keV (Au-Lα) was measured by scanning a 4 μm thick Gold (Au) foil. A 80 mm^2 Sirius 80 Si(Li) semiconductor detector (Grashham, UK) was used to acquire the spectra. Areas of 300 μm × 300 μm to 600 μm × 600 μm were scanned with a