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Spatial distribution of the trace elements zinc, strontium and lead in human bone tissue ☆

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ABSTRACT

Trace elements are chemical elements in minute quantities, which are known to accumulate in the bone. Cortical and trabecular bones consist of bone structural units (BSUs) such as osteons and bone packets of different mineral content and are separated by cement lines. Previous studies investigating trace elements in bone lacked resolution and therefore very little is known about the local concentration of zinc (Zn), strontium (Sr) and lead (Pb) in BSUs of human bone. We used synchrotron radiation induced micro X-ray fluorescence analysis (SR μ-XRF) in combination with quantitative backscattered electron imaging (qBEI) to determine the distribution and accumulation of Zn, Sr, and Pb in human bone tissue.

Fourteen human bone samples (10 femoral necks and 4 femoral heads) from individuals with osteoporotic femoral neck fractures as well as from healthy individuals were analyzed. Fluorescence intensity maps were matched with BE images and correlated with calcium (Ca) content. We found that Zn and Pb had significantly increased levels in the cement lines of all samples compared to the surrounding mineralized bone matrix. Pb and Sr levels were found to be correlated with the degree of mineralization. Interestingly, Zn intensities had no correlation with Ca levels. We have shown for the first time that there is a differential accumulation of the trace elements Zn, Pb and Sr in BSUs of human bone indicating different mechanisms of accumulation.

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Introduction

When tissue of living organisms is analyzed by highly sensitive chemical analytic methods, specific chemical elements in very minute quantities (<ppm) can be found. These so called trace elements can be essential and/or non-essential for the living organism [1]. However, the role of many trace elements in tissues e.g. bone is poorly understood [2]. Great efforts have been undertaken to determine the incorporated amounts of various trace elements in bone [3,4]. Since in general the chemical analysis is based on destructive methods, the information about the spatial distribution of the trace elements within the tissue is usually lost. Previous studies lacked spatial distribution and merely differentiated between cortical and trabecular bone [5–10]. New developments in synchrotron radiation technology allow now analyzing in a non-destructive way, spatially resolved trace elements like zinc (Zn), strontium (Sr) and lead (Pb) in bone tissue. For example using synchrotron radiation induced confocal micro X-ray fluorescence analysis (SR μ-XRF) we found a highly specific accumulation of Pb and Zn in the transition zone between mineralized and nonmineralized articular cartilage compared to subchondral bone [11,12]. Moreover this method is also able to detect and map different elements simultaneously [13].

Zn, Sr and Pb are trace elements, present in sufficient concentrations in bone so they can be easily mapped with the multi-elemental SR μ-XRF method. Zn is an important essential trace element in multiple biological processes and a reduced intake may lead to chronic diseases [14]. Zn is also present in bone tissue and it has been reported to play an important role in bone metabolism [15–17]. Studies on the Zn levels in different tissues revealed that most of it is present in bone and for this reason Zn may be considered as an essential component of the calcified matrix [18,19]. Sr is likely a non-essential trace element, but in recent years, studies have shown that Sr is able to influence bone turnover [20] and has been applied in the form of strontium ranelate in

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therapeutic treatment of osteoporosis. Sr is chemically very similar to calcium (Ca), and can replace Ca, but still little is known about the role of Sr in normal bone metabolism as well as in bone disorders. Pb is a non-essential trace element and represents a highly toxic heavy metal. One of the main threats to human health from heavy metals is associated with exposure to Pb. Exposure to Pb is associated with chronic diseases in the nervous, hematopoietic, skeletal, renal and endocrine systems [21,22]. Pb has been stated also as a potential risk factor for osteoporosis [23] and osteoarthritis [24]. Approximately 95% of the total body Pb burden is stored in skeleton [25] indicating that the bone tissue has a high capacity to accumulate and store Pb. In this context the bone tissue seems to have also the function to keep down the serum levels of such highly toxic elements.

Human bone is essentially composed of a non-homogeneous and non-isotropic arrangement of mineralized collagen fibrils. Cortical and trabecular bones are formed by individual osteons and bone packets (so called bone structural units — BSUs). They are produced at different moments during the (re)modeling cycle by the coordinated activity of bone cells, whereby the osteoblasts synthesize, secrete and deposit the collagenous matrix, which then gradually mineralizes. Thus, each BSU has a certain mineral content depending on the time of deposition [26]. In general these BSUs are connected by a thin layer of mineralized non-collagenous proteins, the so called cement line/layer produced during the remodeling cycle [27]. Only very little data are available regarding the detailed spatial distribution of trace elements within such a bone tissue.

Thus, the aims of this study were to map the trace elements Zn, Sr and Pb in bone tissue and to elucidate the following questions: i) is there a differential accumulation pattern of Zn, Sr and Pb depending on Ca content of mineralized bone matrix in the bone packets, osteons, and interstitial bone? and ii) is the accumulation of Zn, Sr and Pb in cement lines different from that of mineralized bone matrix? Taking into account that the spot size of the confocal SR μ-XRF setup is about 5 times wider than the width of the cement lines the measured intensities are actually a huge underestimate of the real levels of trace elements in this region.

For this purpose we analyzed trabecular and cortical bones from human femoral necks and heads using SR μ-XRF in combination with quantitative backscattered electron imaging (qBEI). qBEI, a well established and validated method [28], was used to visualize the mineralized tissue with a spatial resolution of 1 μm per pixel, to quantify the local bone mineral/Ca content and select the regions of interest for SR μ-XRF measurements in the bone tissue.

Materials and methods

Bone samples

For this study bone samples from 14 postmenopausal women have been analyzed: a) Femoral neck samples (n = 10) which had been part of a former study [29,30] and were kindly provided by N. Loveridge (Department of Medicine, University of Cambridge, Cambridge). Five of these samples were from patients suffering from an osteoporotic femoral neck fracture and 5 samples were from forensic autopsies of individuals without metabolic bone diseases age matched with that of osteoporotic fractures. The average age of these individuals was 81.5 years ranging from 74 to 92 years. b) Femoral head samples (n = 4), which were obtained during hip replacement surgery. The individuals suffered an osteoporotic femoral neck fracture and were 60 to 80 years old with an average age of 77.5 years. Measurements were performed in both trabecular and cortical bone regions for the femoral neck samples and only in the trabecular region for the femoral head samples resulting in a total of 35 areas of about 500 μm × 650 μm. The term mineralized bone matrix will describe both the osteons and the interstitial bone in the osteonal bone region and bone packets in cancellous bone region. To the best of our knowledge, none of the patients has been exposed to higher Pb concentrations than the natural levels in their living areas. The study was in accordance with and approved by the local ethics committee (Institutional Review Board of the Medical University of Vienna).

Sample preparation

As already described in earlier publications [31,32], the samples have been prepared as blocks of undecalcified in polymethylmethacrylate (PMMA) embedded bone tissue. The femoral neck samples were cut in the transversal plane and the femoral head samples perpendicular to the articular surface (frontal plane). The section surfaces were manufactured by grinding with sand paper and subsequently polishing with diamond suspension (3 and 1 μm grain size) on a precision polishing device (PM5: Logitech Ltd., Glasgow, UK) or by milling with a diamond ultra miller (SP2600: Leica Microsystems GmbH, Wetzlar, Germany). The entire embedding and surface preparation procedure was tested to be free of detectable Zn, Sr and Pb contaminations.

qBEI

Quantitative backscattered electron imaging (qBEI) is a validated technique to visualize and quantify the calcium (Ca) concentration distribution in bone based on the backscattering of electrons from the sample surface in a scanning electron microscope (SEM). Areas with bright gray levels reflect matrix with high Ca content, whereas areas with dark gray levels indicate low Ca content. Cement lines, the transition zones between different bone packets and osteons usually show a higher mineral content than the adjacent mineralized bone matrix [26,33]. More details on the qBEI method can be found elsewhere [31,34].

A SEM (DSM 962, Zeiss, Oberkochen, Germany) was employed to acquire qBEI images using 20 keV electrons leading to an information depth of about 1.5 μm [35]. Images at different magnifications 12-fold for overviews and 200-fold (pixel resolution of about 1 × 1 μm^2) were obtained to select and define the region of interest (ROI) in bone for SR-μ-XRF analysis similar to a study done previously [32]. Especially areas (bone packets, osteons) containing mineralized bone matrix with different degrees of mineralization have been selected.

SR-μ-XRF

The properties of synchrotron radiation (SR) including high photon flux, natural collimation, polarization and the possibility to select the energy of the primary photons enabled sensitivities up to the femtogram range and a high spatial resolution in the micrometer range. In previous studies, the combination of a confocal geometry and SR allowed the analysis of trace elements in bone and articular cartilage at the micrometer range with high-sensitivity and high spatial distribution [11,36,37]. Further details on confocal SR-μ-XRF can be found elsewhere [38–42].

The present measurements have been carried out at the FLUO beamline of the ANKA synchrotron facility at the Karlsruhe Institute of Technology Campus North [40,41] applying the same confocal setup as already described previously [32]. The actual excitation energy was 17 keV and the beam size was 17 μm × 12 μm (horizontal × vertical) with a depth resolution of 19 μm at 9.71 keV (Au-Lα). Area scans in the sample surface were performed in the range of 500 μm × 500 μm up to 500 μm × 650 μm with a step size of 15 μm horizontal and 10 μm vertical. Acquisition times longer than 12 s per pixel were found not to show any improvements in the signal to noise ratio of the obtained elemental maps. Especially, the low levels of Pb content required this relatively long acquisition time. The acquired spectra, an example of which is shown in Fig. 1, were processed according to the protocol described in [32].

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