SR-XRF INVESTIGATION OF HUMAN BONE

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ABSTRACT

To investigate mineral and trace elements in human bone the micro-fluorescence facility installed at the bending magnet Beamline L at HASYLAB, Hamburg, was used, focusing our interest on differences in concentrations of lead between the outer (Substantia Compacta: SC) and inner bone region (Substantia Spongiosa: SS). Among mineral and heavy elements, Pb has been proven to exert adverse effects in men with T₁/₂ in human bone that ranges between 6-12 years. Thus, bone of a hip-operated adult patient was sliced perpendicular to the SC/SS transition and investigated. For the Pb detection, excitation with the synchrotron white spectrum to excite the K-shell and a monochromatic radiation obtained with the Si(111) channel cut to measure the L-shell fluorescence was compared. The monochromatic excitation delivered the best peak to background ratio and detection limits in sub ppm range. While the K-shell excitation did not reveal sufficient results, L-shell fluorescence was adequate with detection limits for Pb of 0.8 ppm for white, and of 0.25 ppm for monochromatic excitation respectively. From the medical point of view in the investigated bone sections, Pb, Ca, Fe and Zn were found in higher concentrations in SS than in SC, while Br and Ni, Cu seemed to be lower in the SS than in the SC area.

INTRODUCTION

Mineral and trace elements are not homogeneously distributed in human bone. Differences in concentrations have been documented between the outer (Substantia Compacta: SC) and inner bone region (Substantia Spongiosa: SS) [1]. However, little information is available about the distribution and the concentration ratio of mineral and trace elements within this transition area (SC/SS). X-ray microbeam spectrometry seems to be an adequate device for non-destructive investigations and small spatial resolutions of mineral and heavy elements in bone tissue or other specimen. Using energy dispersive x-ray fluorescence spectrometers (EDXRS) information about different mineral or heavy element concentrations in small areas are possible. Scanning across areas of interest with high flux synchrotron radiation results can be obtained in a reasonable time. Of special interest is the distribution of lead (Pb) which has been proven to exert adverse effects in men [2]. T₁/₂ of Pb in human bone ranges between 6-12 years [3,4]. Only with increased bone metabolism such as hyperthyroidism, Pb excretion is increased. We recently could show that Pb correlates with excreted mature bone collagen as determined by desoxypyridinoline crosslinks.
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Lead in bone is thought to be a parameter for cumulative lead exposure and has thus been investigated in vivo intensively with L-line X-ray fluorescence instrumentations mostly using a $^{109}\text{Cd}$ source [6,7]. But these devices are restricted in their investigations to cortical bone [6]. In this preliminary study mineral and trace elements including Pb were investigated in the SC, SS and SC/SS transition in a bone of a hip-operated adult patient. The study of the Pb distribution in the different regions of the bone are important for a better understanding of the metabolism of Pb. For the Pb detection, the excitation with the synchrotron white spectrum to excite the K-shell and a monochromatic beam obtained with the Si(111) channel cut to measure the L-shell fluorescence are compared.

**EXPERIMENTAL SETUP**

The sample was analysed at the micro-fluorescence facility installed at the bending magnet Beamline L at HASYLAB, Hamburg [8]. A GeHP detector with a FWHM of 160eV@5.9keV was used in all measurements. To shield the detector against high energy photons a 3mm wall thickness Pb tube was mounted around the Al end cap of the detector. In the center bore of the Pb cylinder in front of the Be window a Ta disc with a 2 mm pinhole was placed as collimator allowing the fluorescent x-rays to pass and suppressing the Pb lines from the shielding. First experiments were made using a white beam with photon energies up to 300 keV to excite the Pb K lines. The results did not show sufficient signal above the high background. Therefore the monochromatic synchrotron radiation after passing a Si(111) channel-cut single crystal was used. The excited area was defined by vertical and horizontal Ta slits in the beam path right before the sample, set to 400(v) x 500(h) μm². Two different excitation energies were chosen: 15.5 keV to have an optimal excitation of the Pb L lines, 19.5 keV in order to extend the range of elements that could be detected up to Nb.

![Figure 1: Experimental set-up for micro-fluorescence at HASYLAB](image)

**RESULTS**

A piece of hip bone from an operated adult patient was cut to show a plane perpendicular to the SC/SS transition surface. Four linear scans were carried out, two of them over the transition
border SS / SC (A). One linear scan was done on the compact bone (B) and one on the spongy bone to see if the concentration would remain constant in the region (C), see Fig. 2.

Fig. 2 Scheme of the bone section investigated, the letters indicate the 3 scans performed.

Fig. 3 shows the result of the 2 line scans A from SC-SS, with the relative intensities achieved after normalization to the maximum intensity of the respective element within the scan. Only the synchrotron current corrected intensities were taken.

In the graphical representation in Fig. 2 the scans begin on the Substantia Compacta (the outer part of a bone) and end in the Substantia Spongiosa (the inner part of bones).
Figure 3: Graphical representation of the measurements. The intensities for each element in one scan have been normalised to the maximum intensity value of the respective element within the scan. The maximum of obtained net counts in respectively 800sec (upper scan) and 1000sec (lower Scan) is reported in the legend.

The intensities for each element present in the two scans show the same behaviour for some elements. Ni, Cu and Br are correlated and in higher concentration in the SC part. Ca, Fe, Zn and Pb form another group of elements which behave in the opposite way. Se and Ti seem to be constant over the transition. Se was only found in one of the two scans.

Fig. 4 shows a spectrum from scan A, excited with 15.5 keV and a spectrum from scan C excited with 19.5 keV.
Fig. 4 Spectrum A, excited with $E=15.5$ keV, measured for 1000 s.
Spectrum from scan C, excited with 19.5 keV, measured for 1000 s.

Fig. 5 shows the scan in the substantia compacta.

Fig. 5 Scan in the substantia compacta, intensities normalized to the maximum intensity of the respective element in the scan. The maximum of obtained net counts in 1000 sec is reported in the legend.

Figure 6 shows the scan in the substantia spongiosa:
DISCUSSION

The preliminary results of our x-ray micro-beam spectroscopy at the bending magnet beam-line L, HASYLAB, in Hamburg show interesting physiological and physical aspects. From the physiological point of view we could demonstrate that Pb was found in higher concentrations in the SS than in the SC compartment. This finding is in agreement with earlier investigations where Pb has been determined by flameless atomic absorption spectroscopy [1]. Additionally we found that the distribution profile for Ca and Zn was in parallel with Pb. In a recent paper we suggested new aspects of anaemia induced by lead, and could show that bone marrow progenitor cells (e.g. erythroid burst forming units (BFU-E)) are dose dependent reduced by lead [9]. Since the SS compartment is in direct contact with the bone marrow and the metabolism in SS is faster than in the SC compartment our findings would support our new aspect of lead induced anaemia.

Furthermore Zn was found in higher concentrations in SS and as shown recently an increase of Zn porphyrins can lead to an apoptosis of BFU-E cells [9]. On the other hand it seems not surprising that investigations of in vivo measurements of tibia lead concentrations after chelation therapy did not show significant alterations of bone lead, since in vivo measurements consider only cortical lead concentrations [1]. Thus from the physiological point of view micro-beam spectroscopy can not only be helpful in analyzing mineral and trace elements in bone but can also be helpful in the interpretation of patho- and physiological phenomena.

From the physical point of view it was unexpected, that with linear polarized radiation of high intensity and high energies the K-shell excitation of Pb failed. In the L–line region the determination of Pb was possible in both spectral modes – white and monoenergetic, with better conditions as concerns signal, background and detection limits. The chosen size of the beam spot 400µm vertical and 500 µm horizontal was enough to give sufficient spatial resolution in the

Fig. 6 Scan in the substantia spongiosa, intensities normalized to the maximum intensity of the respective element in the scan. The maximum of obtained net counts in 1000sec is reported in the legend.
transition region and with this illuminated area, detection limits where about one order of magnitude below the expected concentration values of Pb in bone 3-8 µg/g.

FUTURE

The interesting results encourage further investigation. With the aid of capillary optics, analyses with spatial resolution of about 20µm x 20µm are planned. Multilayer monochromator will give a higher flux and therefore shorter measuring time. The resolving power of a Si(111) monochromator could be used for a XANES investigation of the Pb LIII edge in the sites suggested by the spatial scans. A Si(Li) is preferred to avoid the strong overlapping escape lines.

REFERENCES