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Measurement of the microdistribution of strontium and lead in bone *via* benchtop monochromatic microbeam X-ray fluorescence with a low power source

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Summary

Determination of the microdistribution of trace elements in bone at low concentrations has previously been performed with proton induced X-ray emission (PIXE), high-energy synchrotron source X-ray fluorescence (XRF) and laser ablation - inductively coupled plasma mass spectrometry (LA-ICP-MS). Several commercial benchtop XRF systems with micrometer-scale resolution are currently available. While providing convenient, non-destructive mapping capability, they appear to lack the sensitivity required for detection of trace elements in biological tissues such as bone. We investigated the application of a prototype benchtop XRF system for the measurement of strontium and lead at physiological levels in bone. Detection of several elements of interest, including Sr and Pb was achieved with an experimental set up based on focused monochromatic microbeam X-ray fluorescence (M μ -XRF) instrumentation with a low power source (45 W molybdenum tube) coupled to doubly curved crystal (DCC) optics. A cross-section of bone about 5 mm $\times$ 8 mm size was mapped with 80- μ m resolution showing heterogeneous distribution of Sr and Pb. The data showed that M μ -XRF coupled to DCC is powerful method for measurement of the spatial distribution of trace elements in bone.

1. Introduction

Measurements of the spatial distribution of lead in bone have previously been made by proton induced X-ray emission (PIXE),^{1,2} high-energy synchrotron X-ray fluorescence (XRF),^{3–5} and laser ablation - inductively coupled plasma - mass spectrometry (LA-ICP-MS).⁶ PIXE provides relatively high limits of detection, in the $\mu\text{g g}^{-1}$ range. Synchrotron-based XRF has lower limits of detection, but the limited availability and high cost of the synchrotron source are somewhat prohibitive for experimental studies. LA-ICP-MS is well established for laboratory analysis and has been widely employed for biological materials,⁷ since limits of detection in the ng/g range can be achieved. However, LA-ICPMS requires skilled optimization and operation, and suffers from stability issues and element fractionation.^{8,9}

Several XRF instruments are commercially available that have the capability for mapping at micrometer-scale resolution. They are relatively simple to operate, but appear to lack the necessary sensitivity for work at the low concentrations required for the analysis of trace elements in biological samples (i.e., ng/g). We obtained a demonstration of the Horiba XGT 5000 XRF microprobe, which uses a monocapillary guide tube to obtain micrometer scale resolution, but the limits of detection proved inadequate determination of Pb and Sr in our samples of interest.

Recent advances in X-ray optics coupled to monochromatic energy dispersive X-ray fluorescence (M μ -XRF) have shown great potential for high-definition XRF mapping.^{10,11} The system has demonstrated maximum detection limits at femtogram levels, and spatial resolutions better than 100 μ m, for 2 μ L droplets placed on silicon wafers; it thereby provides a significant improvement in performance over other benchtop X-ray fluorescence systems with. The improved performance results from the use of a doubly curved crystal optic (DCC) (X-ray Optical Systems (XOS) Inc, East Greenbush, NY) to focus the X-rays, and thus increase the usable flux (1×10^9 photons/s) from a standard laboratory source.^{13,14} Signal to background ratios (i.e., the specific element peaks above Bremsstrahlung continuum) are substantially improved.¹⁰ M μ -XRF with DCC thus provides a new opportunity for determining the spatial distribution of both major and trace elements at micrometer-scale resolution in solid materials, including biological tissues.

The aim of this work was to determine, through the analysis a sectioned of goat tibia, whether the M μ -XRF system could be successfully applied to determination of the microdistribution of trace elements in bone.

2. Materials and Methods

Sample preparation

A goat tibia expected to contain about 10 μ g g⁻¹ Pb was selected from a repository of bones maintained by the New York State Department of Health's Wadsworth Center. The source of the bones in the repository is a herd of goats and cows managed by the Wadsworth Center. Each year, several goats are dosed with small amounts of lead acetate to produce blood physiologically enriched in lead (i.e., lead is metabolically incorporated into red blood cells) for use in the NYS Proficiency Testing (PT) scheme for blood lead.^{15,16} These activities are conducted according to a protocol approved by the Center's Institutional Animal Care and Use Committee. The Wadsworth Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, has an approved Animal Welfare Assurance (#A3183-01) with the Public Health Service, and is registered as a Class R Research Facility (#21-R-0124) with the United States Department of Agriculture. As animals near the end of their natural life times, the Center's Facility Veterinarian will recommend euthanasia. In such cases, arrangements are made to collect various organs and bone samples post mortem, which are stored at -80°C for research purposes.

A bone from the repository was physically scraped clean using tantalum tools and treated with hydrogen peroxide solution (30% w/v) to remove any residual tissues and marrow. The bone was initially sectioned near the midpoint of the shaft using a rotary sawing tool fitted with a diamond disc wheel (Dremel, Racine, WI). A flat, smooth surface was then prepared with an Isomet slow speed diamond disc saw (Buehler Ltd, Evanston, IL). Prior to analysis, the surface was etched in dilute hydrochloric acid. Other sections of the bone were retained for comparative analysis via acid digestion followed by determination of Pb and Sr by ICP-MS.

ICP-MS

The bulk concentrations of Sr and Pb in the bone were determined by ICP-MS. Sections of the tibia were sampled adjacent to the section retained for μ XRF analysis. After drying in a vacuum desiccator for 24 h (yielding 1–2 g dry weight), samples were digested overnight in 5 mL concentrated nitric acid at room temperature, in sealed 50-mL tubes. After a 100-fold dilution, the solutions were analyzed using a Model DRC plus ICP-MS (Perkin-Elmer, Shelton, CT)[†]. Instrument parameters were optimized on the day of analysis *via* the manufacturer's standard protocol. External calibration standards were prepared from commercially available stock solutions. Standards and samples contained 100 μ g/L Y and 10 μ g/L Bi as internal standards for Sr and Pb, respectively. The masses ⁸⁸Y, ⁸⁹Sr, ²⁰⁶Pb, ²⁰⁷Pb, ²⁰⁸Pb, and ²⁰⁹Bi were measured in standard mode (i.e., no DRC gas). Certified reference materials developed in this laboratory¹⁷ were analyzed for quality control purposes. The analytical run was accepted if the determined value agreed with the certified value within the measurement uncertainty of the method.

μ XRF-DCC

A prototype μ XRF-DCC system equipped with an energy-dispersive detector was assembled by XOS, East Greenbush, NY. After optimization, an area of the bone section measuring about 5.0 mm $\times$ 8.0 mm was analyzed by energy-dispersive μ XRF. The energy dispersive μ XRF arrangement is shown in Figures 1 and 2. The X-ray optic system, consisting of an X-ray source and a doubly curved crystal (DCC) optic, provides a small spot monochromatic beam. The Mo K α X-ray beam 17.48 keV was chosen as the excitation source. The beam was focused onto a 60- μ m spot size by the DCC optic (the DCC optic diffraction energy is designed at 17.48 keV). A silicon drift detector (SDD), produced by SII NanoTechnology USA Inc., was used for recording the resultant X-ray fluorescence spectra at selected positions located on the sample surface. The system was mounted on a table of 150 $\times$ 150 mm² equipped with X-ray safe protection and safety interlocks. Each sample was mounted on a holder, which was attached to a stage translatable along x, y, and z axes. The sample was aligned at the beam focal spot such that the surface was parallel to the scanning directions, i.e., in the x-y plane. The scanning spatial resolution was 80 μ m. For each site, the count-time was 25 s. In total, 6,300 sites were analyzed over a period of 44 h, to accumulate sufficient data points for high resolution mapping. The analysis was fully automated and unattended.

The data output was arranged into a table giving the X-ray intensity of specific X-ray peaks representing element signals each measured point defined by its X and Y coordinate (mm). Element intensities were normalized to the scattering peak arising from the bone matrix. A table of the X and Y coordinates and normalized element intensities (Z) was copied into a scientific graphing and analysis software package (Origin version 7.5, OriginLab Corporation, Northampton, MA). The data were converted using the software's function into a data matrix, from which XY(Z) contour maps (*2-dimensional maps*) of the data were generated.

3. Results

Figure 3 shows the cross section of the measured goat tibia, the red box indicating the approximate analyzed area on the anterior side of the bone. Similar goat bones from our repository were determined to have a dry ash weight, indicating the mineral to organic ratio, of about 72 wt% (after ashing at 450°C for 24 h). The calcium content of similar bones was

[†]Certain equipment, instruments or materials are identified in this work to specify adequately the experimental procedure. Such identification does not imply a recommendation or endorsement by the New York State Department of Health.

about 27 wt% (dry weight). Sr and Pb concentrations from an adjacent section of this tibia, determined by ICP-MS following acid digestion, were $115 \pm 3 \mu\text{g/g}$ (dry weight) and $10.1 \pm 0.2 \mu\text{g/g}$ (dry weight), respectively. Typical Sr concentrations in human bone are about 250 $\mu\text{g/g}$ (dry weight).¹⁸ Typical bone Pb concentrations in humans range from about 1 to 80 $\mu\text{g/g}$ (dry weight) (or greater, in cases of chronic exposure to high levels of lead).¹⁹

Figure 4 shows a typical X-ray spectrum from a single measurement site on the tibia surface (located near the center of the measured area). It is evident from the $K\alpha$ and $K\beta$ lines for Ni, Cu, Zn, Cu, Sr, Br, and Pb that these elements are clearly detectable in the bone with this $\text{M}\mu\text{XRF}$ system. We focused our attention on Sr ($K\alpha$ peak) and Pb ($L\alpha$ peak), since these elements have known biological significance in bone, as discussed later in this section. While atmospheric contamination by Sr and Pb in the laboratory cannot be entirely discounted, we saw no evidence that this had occurred. K-alpha ($\sim 10.5 \text{ keV}$) produces an interference with the Pb L-alpha. There is no evidence of significant As in the sample ($< 0.1 \mu\text{g/g}$ dry weight), and it is thus likely that this interference affects the Pb signal.

The elastic scatter recorded over the mapped area (Figure 5) varied significantly, due to the geometry of the source-sample-detector arrangement. The scattering peak results from the interaction of incident photons with Ca, P and other elements in the bone matrix with relatively high atomic numbers (elements with low atomic numbers, such as C, contribute relatively little to the scattering peak). The intensity of the scatter is thus directly proportional to the amount of bone mineral matrix excited by the X-rays. Absolute elemental signals recorded by the detector vary in same same fashion to the scatter, independently of their concentration, so normalization of raw signal intensities to the mean scattering intensity is appropriate. Figure 6 shows the mapped intensities of Sr and Pb following normalization. The plots show the total counts per unit of bone mineral. Intensities below the limit of quantitation (LOQ), calculated as 10 standard deviations of the background signal, were assigned a count concentration of zero.

Strontium showed a striking distribution, with two enriched concentration bands near the center of the core, and lower levels towards the periosteal (outer) and endosteal (inner) surfaces. Strontium is thought to be important in bone health.²⁰ Recently, therapeutic drugs containing strontium have shown promise in the prevention and treatment of osteoporosis²¹. The proposed mechanism is that Sr accumulates at the bone surface, preventing fracture and resorption.^{22–23} $\text{M}\mu\text{XRF}$ would clearly be a powerful technique for future studies in this area.

Lead appeared to accumulate in a thin band near the periosteal surface. Elevation in scattered discrete spots was also observed at the endosteal surface, and to a lesser extent within the core. Enriched lead at the periosteal surface has previously been observed in human tibiae, after physical separation of surface and core, followed by acid digestion and atomic absorption spectrometry analysis.²⁴ A similar enrichment was observed in other goat bones from our repository, analyzed by the similar methods (Y. Y. Zong, PhD Thesis, School of Public Health, University at Albany, Albany, NY). Enrichment of lead at the periosteal surface may be significant, inasmuch as it increases the likelihood of lead remobilization during bone demineralization. Also, lead accumulation at the surface of the bone may affect non-invasive measurement of lead in bone via *in vivo* XRF methods,²⁵ since lead in the surface bone has a higher probability of detection than does lead located in the core.

Elemental concentrations may be calculated from future $\text{M}\mu\text{-XRF}$ data *via* external calibration using appropriate standards, or using calculations based on fundamental parameters. Here, we can estimate the Pb and Sr concentrations using the ICP-MS data

described above, assuming that these data faithfully represent the mean concentration present in the M μ -XRF section. Table 1 summarizes the sensitivity coefficients calculated as the ratio of the mean normalized M μ XRF counts to the mean ICP-MS concentration. Using these coefficients, the limit of detection (LOD) was estimated based on three times the standard deviation of the blank counts. The minimum and maximum concentration of Sr and Pb in the bone was also calculated, based on the contoured bands as shown in Fig 6 rather than from individual measurement sites. The LOD for Pb in bone by M μ -XRF is 0.5 μ g, whereas the LOD for Pb in bone by LA-ICP-MS is 0.2 μ g/g based on matrix-matched external calibration.⁶ It should be noted, however, that the M μ -XRF LOD was calculated from background counts in the vicinity of the tibia, whereas the LA-ICP-MS LOD was calculated from the analysis of a bone sample with background concentrations of Pb.

4. Conclusions

M μ -XRF with a low power source coupled with DCC optics demonstrated excellent detection capability and stability for determination of trace elements with micrometer-scale resolution in bone. Potential advantages of this system compared to alternative methods such as synchrotron-based XRF and LA-ICPMS are its potentially low costs and simple, automated operation.

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Figure 1. Schematic of Mμ-XRF arrangement showing positioning of the doubly curved crystal optic (DCC) and other components, as used for the analysis of the bone sample.

Figure 2.
Mu-XRF doubly curved crystal optic (DCC) set up.

Figure 3.
Photograph of the bone sample. The red box indicates the approximate analyzed area.

Figure 4. X-ray spectrum recorded by M μ -XRF with DCC near center of tibiae (core). The main element peaks are labeled accordingly.

Figure 5.
Intensity of elastic scatter by M μ -XRF. The periosteal (outer) surface is located towards the left margin and the endosteal (inner) surface is located towards the right margin.

Figure 6.

Distribution of Sr and Pb in the bone sample measured by μ -XRF after normalization of the raw elemental signals to mean scatter. The periosteal (outer) surface is located towards the left margin and the endosteal (inner) surface is located towards the right margin.

Table 1

Estimated sensitivity coefficients and summary statistics for M μ -XRF analysis of tibia for Pb and Sr. Concentrations are reported based on dry weight.

	Strontium	Lead
Mean concentration in tibia section [*] ($\mu\text{g/g}$)	115	10.1
Mean normalized M μ -XRF intensity in tibia section (counts)	3368	245
Sensitivity coefficient (counts/($\mu\text{g/g}$))	29.2	24.3
Limit of detection ^{**} ($\mu\text{g/g}$)	1.1	0.5
Min.-Max concentration in tibia section ($\mu\text{g/g}$) ^{***}	52 – 142	5.9 – 23.6

^{*} as determined by ICP-MS

^{**} three times the standard deviation of the blank areas

^{***} As defined from contour bands in Fig. 6.