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Elemental distribution in human femoral head

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ABSTRACT

Osteoporosis is the most common bone disease with severe symptoms and harmful effects on the patient quality of life. Because abnormal distribution and concentration of the major and trace elements may help to characterize the disease, ion beam analysis is applied to the study of bone samples. Proton Induced X-ray Emission and Elastic Backscattering Spectrometry are applied for qualitative and quantitative analysis of an osteoporotic bone sample, for the determination of the Ca/P ratio and analysis of the distribution of major and trace elements. The analysis was made both in trabecular and cortical bone and the results are in agreement with the information found in literature.

1. Introduction

Osteoporosis affects elderly people and has severe symptoms with harmful effects in the patient's quality of life. Osteoporosis is a major public health threat affecting more than 200 million people worldwide [1]. In Portugal the disease affects 5% of the population [2]. This skeletal disorder is characterized by bone strength loss and micro-architectural deterioration of bone tissue, increasing the risk of fractures. In osteoporosis bone formation is slower than bone resorption, and consequently the trabecular plates have increased perforations and there is loss of trabecular connectivity [3,4].

Healthy bone is a mineralized connective tissue composed by water, an organic phase (mainly collagen type I) which surrounds the mineral crystals, and an inorganic part best approximated as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), that makes about a quarter of the bone volume and 60–70% of dry weight of adult normal bone. The fraction of calcium in hydroxyapatite is 39.9%, phosphorous 18.5% and the Ca/P ratio is ~2.2. [5,6]. Hydroxyapatite may contain a number of impurities such as HPO_4^{2-}, CO_3^{2-}, Mg^{2+}, Na^+, F^-, which are absorbed by the crystal or may replace the Ca^{2+}, PO_4^{3-} and HO^- ions. The relative content of Ca and P is critical for sustaining mineral homeostasis and bone metabolism and it is considered a suitable biomarker for the assessment of bone health [7].

Morphological abnormalities in osteoporosis may induce modifications of the elemental concentrations and of the distribution of major and trace elements in the bones. In addition, an excessive or a diminished concentration of a specific element may trigger metabolic alterations that lead to the disease. In this work, ion beam analysis techniques like Proton Induced X-ray Emission (PIXE) and Elastic Backscattering Spectrometry (EBS) are applied to identify and determine the elemental concentration of the elements on osteoporotic bone and to do elemental 2D mapping on a specific region of the bone.

Previous authors applied several techniques to the analysis of bone samples, namely Synchrotron Radiation X-ray Fluorescence (SR-XRF) and μ-PIXE, to perform quantitative and qualitative analysis of bone samples to elucidate the basic mechanisms underlying the initiation and developmental changes that are associated to bone diseases [8–11]. These two techniques have similar spatial resolution and, although μ-SR-XRF has marginally better elemental detection limits, μ-PIXE has the main advantage of quantification of the elements without standards and is a much more cost/effective and available technique for elemental analysis at the ppm level.

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2. Materials and methods

2.1. Sample preparation

To obtain bone samples, collaboration with national orthopedic services is required and this is only possible with formal assent of the ethics committee of the respective hospital. After an informed consent from patient, the bone samples are collected, during surgery, when (due to clinical causes and accidents) it is necessary to remove small pieces of bone, which occur in the majority of orthopedic surgeries. The bone sample analyzed was resected during a hip replacement surgery of an 86 years old female patient. The thin sample of osteoporotic human femoral head was cut using a water-cooled SiC saw, to obtain a slice ~2 mm thick. This section was subsequently heated in water for a few minutes and then polished using silicon carbide paper and a continuous slow flow of water to prepare a slice ~1 mm thick.

2.2. Experimental conditions

The bone was analyzed, under vacuum conditions, using a 2.0 MeV proton focused beam ($4 \times 5\ \mu m^2$) produced by the 2.5 MV Van de Graaff Accelerator of the CTN/IST, using the nuclear microprobe (Oxford Microbeams type) [12]. OMDAQ software was used for collecting and processing data from the nuclear microprobe, in listmode (or event-by-event) data collection. The 2D elemental maps have 256 × 256 pixels. The experimental chamber holds a 80 mm^2 Link X-ray detector (145 eV energy resolution, positioned at 135° to the beam direction) and a Passivated Implanted Planar Silicon (PIPS) detector (positioned at 140° to the beam direction) to register simultaneously the photon signals and the backscattered protons coming from the sample, respectively. The 2D elemental maps shown in Fig. 1 were obtained in routine operation mode where a 50 μm Mylar filter is used in front of the Si(Li) detector for preventing backscattered protons from entering into the detector. The use of this filter however implies a high intensity signal from the Ca K lines and then poor detection limits for high Z elements in a reasonable amount of time. To enhance trace elements detection limits, some spectra were obtained using a 1 mm thick Perspex filter. PIXE spectra evaluation and quantification was done with the GUPIX [13], and for EBS spectra analysis, WinDF code was used [14].

3. Results

A selected area of the sample was scanned with the proton micro-beam ($2640 \times 2640\ \mu m^2$), and the images of the elemental distributions for Ca, P and Fe were produced (Fig. 1).

The beam was positioned in a region near the border of the bone to allow a simultaneous analysis of the two different types of bone tissue, trabecular (1) and cortical (2) bone. The areas chosen to perform quantitative analysis (and results presented in column 1 and 2 of Table 1) are identified by the white circles. The maps show that the distribution of the two major elements (Ca and P) is not homogeneous among cortical and trabecular bone, with the cortical bone having higher concentration of calcium and phosphorous. This is one of the reasons why trabecular bone is more susceptible to fracture in osteoporosis. Figs. 2 and 3 pertain to the PIXE spectra obtained in these two regions: trabecular and cortical bone, respectively. Besides calcium and phosphorous it

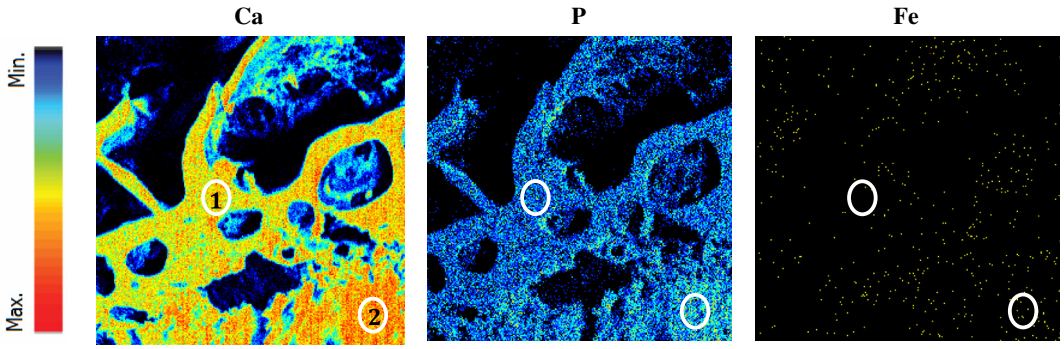

Fig. 1. 2D elemental distribution of Ca, P and Fe along the bone section ($2640 \times 2640\ \mu m^2$).

Table 1
Concentration of the elements identified on the PIXE spectra recorded in the trabecular bone (PIXE spectrum 1) and different parts of the cortical bone (PIXE spectrum 2 and 3). The concentrations of bromine, strontium and copper for trabecular bone were not accessed (n.a.). The uncertainties showed are just the combined errors calculated with the values of fit error and experimental error coming from the GUPIXFIT output.

	PIXE spectrum		
	1 Trabecular tissue	2 Cortical tissue	3 Cortical tissue (Perspex filter)
Major elements (%w/w)			
Ca	16.9 ± 0.4	33.6 ± 0.3	–
P	4.0 ± 1.0	12.5 ± 0.7	–
Trace elements			
S	269 ± 63	873 ± 122	–
Zn	77 ± 14	262 ± 28	250 ± 3
Cl	95 ± 22	321 ± 40	–
Fe	–	148 ± 16	271 ± 3
Br	n.a.	–	5 ± 1
Sr	n.a.	–	36 ± 3
Cu	n.a.	–	8 ± 1