Nuclear Instruments and Methods in Physics Research B 279 (2012) 223–226

Contents lists available at SciVerse ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb

Major and trace elements in mouse bone measured by surface and bulk sensitive methods

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ARTICLE INFO

Article history:
Received 22 July 2011
Received in revised form 13 October 2011
Available online 23 November 2011

Keywords:
Mouse bone
Major and trace elements
SEM
PIXE
SNMS
XPS

ABSTRACT

We present experimental investigations of the mouse bone in its original form and after calcination. We used various techniques, like scanning electron microscopy, secondary neutral mass spectroscopy, particle induced X-ray emission, and X-ray photoelectron spectroscopy. Among the major element's concentration profiles we also determined the observable trace elements of the mouse bone.

1. Introduction

In the past years an increasing research interest turned to the accurate determination of the components of bone samples [1–14]. These investigations focused on both the major and trace elements in the bone. Work in this field is strongly motivated because various major and trace element concentrations can be good indicators of several diseases. Number of studies also focused on the determination of the components both in the organic and inorganic parts of the bone separately, because they both have role during bone remodeling processes. Also important to note that bone can be one of the final destinations in the body where toxic elements are deposited.

Dual X-ray absorptiometry is currently the standard method for determining bone mineral density in the general human population. It provides information of the changes in total bone mineral content, but not the distribution of the special elements or the type of underlying disease. Elemental mapping has been at early stages under development for human application [15]. It would be very useful if we would be able to evaluate changes of bone minerals more detailed separately. But until now there are only some contradictory data in animal models and human biopsies [16].

Although the main components of the bone are known for a long time, only recently do we have the insight to the controls of bone formation and mineralization and still little is known about skeletal aging [5]. Basic multicellular units including osteoblasts and osteoclasts, which move in tandem, and osteocytes altogether conduct mineralization of the extracellular matrix. Defective bone mineralization in a variety of inborn and acquired diseases results in osteomalacia, rickets or osteoporosis. Osteoporosis is a systemic bone disease, which is characterized by a generalized reduction of the bone mass. It is the main cause of fractures in the elderly. To make a correct medical diagnosis visual signs in X-rays and bone densitometry by dual energy X-ray absorptiometry are used [6]. These methods provide indirect signs about mineral content of the bone and the special inorganic composition remain hidden. Many nutrients influence bone mass and prevent osteoporosis [7]. Role of the calcium and vitamin D is well-known. Magnesium deficiency also induces osteoporosis [8]. These findings suggest that both calcium and magnesium may have a role in regulation of bone mineralization. Opposite to the effects of calcium and magnesium, inorganic pyrophosphate inhibits bone formation [9]. Accurate determination and follow-up changes in the mineral components of bone samples would be required.

The aim of the present work is to investigate the major and trace elements in mouse bone. We take the advantage of our institute

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0168-583X/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.nimb.2011.10.059

(Atomki) that we have number of techniques available for these studies. We used the followings: scanning electron microscopy (SEM), secondary neutral mass spectroscopy (SNMS), particle induced X-ray emission (PIXE) and X-ray photoelectron spectroscopy (XPS).

2. Sample preparation

The present study conforms to the European Community guiding principles for care and use of laboratory animals. The experimental protocol has been approved by the National Ethics Committee for Animal Research under the reference number of the University of Debrecen (4/2011 DEMAB). Femurs of 20-week-old healthy mice (BDF1, National Institute of Oncology, Budapest) were used.

Mice were exterminated by cervical dislocation, then both femoral bones were removed. They were cleaned from lipids, muscles and tendons. The bone marrow was washed out with distilled water many times through a fine needle syringe. After this the bones were dried for a day and one of the femurs of the mouse was used for analysis in this natural form (see Fig. 1). We note, that scanning electron microscopy may reveal final trabecular structure, which mirrors well the bone quality [10]. The other femoral bones of the mice were calcinated to prepare bone powder. Calcination is known as a process of high temperature heating in the presence of atmospheric oxygen. The end product being pure bone mineral, a compound related to hydroxyapatite. All organic material is combusted to CO_2. The bones were burnt by Bunsen-burner for 10 min, by that time they became snow-white. The porous inorganic bones were broken in a small ceramic mortar.

3. Experimental techniques

3.1. SNMS

Ion-beam sputtering is a widely used method for surface modification [17,18]. During sputtering, the sample surface is bombarded by a low- or medium-energy ion beam. The primary ions of the beam suffer collisions with the surface and induce atom–atom collisions in the target. As a result of an atomic collision cascade, the primary ions are backscattered from the target or stopped in it. If some target atoms near the surface acquire high enough energy from the kinetic energy of primary ions to overcome the surface barrier energy, they are emitted in the form of either secondary ions or neutral atoms.

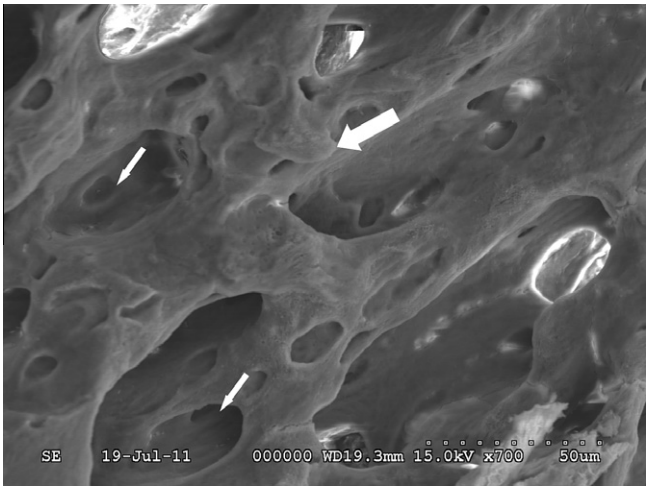

Fig. 1. Scanning electron microscopy image of mouse trabecular bone from femoral diaphysis before calcination. Narrow arrows show the plate-rod structure with fenestrations and wide arrow shows a microtrauma followed by callus formation.

The emitted secondary ions, being in negatively or positively charged state, are analyzed by secondary ion mass spectrometry (SIMS), the emitted secondary neutral atoms and molecules are analyzed by secondary neutral mass spectrometry (SNMS). SNMS is one of the most efficient methods to determine the composition and depth distribution of atomic elements [18]. The analysis of the samples was performed by the INA-X type SNMS system produced by SPECS GmbH Berlin. This system uses low-pressure radio frequency plasma for both sputter and post-ionization. The sample surface is bombarded with the ions extracted from the plasma (Direct Bombardment Mode) by applying high frequency voltage between the sample and aperture (High Frequency Mode) to enable charge compensation. The bombarding ion energy ranges from 100 eV up to 2 kV. The detection system is a quadrupol mass spectrometer for the 1–340 amu range, supplemented with a secondary electron multiplier. The detection limit is 1 ppm. The maximum analyzable area is 14 mm in diameter. Due to the highly uniform sputtering current density over the entire analyzed area and the low primary ion energy (a few hundred eV), a depth resolution of 1–2 nm can be achieved supposing that the sample surface is smooth enough.

3.2. PIXE

PIXE is a nondestructive method for elemental analysis of major, minor and trace elements [19]. We have used 2 MeV proton beam which was focused to 1 μm, and scanned over the samples. The information depth depends on the beam energy, the sample composition, and the emitted X-ray self absorption in the sample which is taken into account during spectra evaluation. But typically we can say that the information depth is in the order of a few tens of microns. Thus a very small piece of the bone was sufficient for the analyses. Typical beam current was about 200 pA, and the spectra were collected for 30–90 min in various places over the samples.

For the collection of X-rays two Si (Li) detectors were used simultaneously. One of them was equipped with a Be window and a 125 μm kapton filter. This attenuates the low energy X-rays, thus the detector was able to detect the heavier trace elements. The other Si (Li) detector had an ultra thin window (UTW), thus sensitive for the low energy X-rays: we were able to see the carbon K-line. This setup is described in more detail elsewhere [20]. The PIXE spectra were evaluated by our home-made software called PIXEKLM [21,22]. The reliability of the setup was successfully tested on various samples [23,24].

3.3. XPS

XPS is an analytical technique to determine the elemental composition and the chemical states of various specimens. In conventional XPS the excitation happens with X-ray tubes emitting narrow line width characteristic X-rays like Al and Mg resulting 0.9 and 0.8 eV line widths at the half maximum of the K_α lines, respectively. The K_α X-ray energies are 1486.67 eV for Al and 1253.67 eV for Mg. These low energy excitation sources produce low kinetic energy electrons from the solid sample. So this technique is very surface sensitive. The information depth in the Mg and Al excited XPS is a few nanometers.

In the present case, photoelectron kinetic energies from a few square millimeter area of bone powder were measured by the help of ESA-31 homemade [25] electron spectrometer. The specimen was prepared from calcinated mouse bone and fixed onto 3M-9703 type double sided conductive sticky tape. We used a fixed retardation ratio mode of the spectrometer with the relative energy resolution of about 1.25×10^{-4}. The measurements were performed with Al anode X-ray tube (15 kV, 10 mA). The basic vacuum level in the measurement chamber (without the bone specimen)