Materials Science and Engineering C 74 (2017) 207–218

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Synchrotron-based XRD from rat bone of different age groups

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ARTICLE INFO

Article history:
Received 23 May 2016
Accepted 29 November 2016
Available online 3 December 2016

Keywords:
Synchrotron
X-rays
Diffraction
Rat bone
Different age groups
Phase
Crystal structure
SEM

ABSTRACT

Synchrotron-based XRD spectra from rat bone of different age groups (w, 56 w and 78w), lumber vertebra at early stages of bone formation, Calcium hydroxyapatite (HAp) [$Ca_{10}(PO_4)_6(OH)_2$] bone fill with varying composition (60% and 70%) and bone cream (35–48%), has been acquired with 15 keV synchrotron X-rays. Experiments were performed at Desy, Hamburg, Germany, utilizing the Resonant and Diffraction beamline (P9), with 15 keV X-rays ($\lambda = 0.82666\ A^0$). Diffraction data were quantitatively analyzed using the Rietveld refinement approach, which allowed us to characterize the structure of these samples in their early stages. Hydroxyapatite, received considerable attention in medical and materials sciences, since these materials are the hard tissues, such as bone and teeth. Higher bioactivity of these samples gained reasonable interest for biological application and for bone tissue repair in oral surgery and orthopedics. The results obtained from these samples, such as phase data, crystalline size of the phases, as well as the degree of crystallinity, confirm the apatite family crystallizing in a hexagonal system, space group $P6_3/m$ with the lattice parameters of $a = 9.4328$ Å and $c = 6.8842$ Å (JCPDS card #09-0432). Synchrotron-based XRD patterns are relatively sharp and well resolved and can be attributed to the hexagonal crystal form of hydroxyapatite. All the samples were examined with scanning electron microscope at an accelerating voltage of 15 kV. The presence of large globules of different sizes is observed, in small age groups of the rat bone (8w) and lumber vertebra (LV), as distinguished from, large age groups (56 and 78w) in all samples with different magnification, reflects an amorphous phase without significant traces of crystalline phases. Scanning electron microscopy (SEM) was used to characterize the morphology and crystalline properties of Hap, for all the samples, from 2 to 100 μm resolution.

1. Motivation

XRD spectra from bone (humans and animals) are studied extensively in a number ways to extract new and novel information about the Ca/P ratio and over the years, calcium hydroxyapatite (HAp) [$Ca_{10}(PO_4)_6(OH)_2$], has been used in biomedical engineering as a coating material in bone implants, to focus on interfacial reactions of a biological system and prosthesis for dental and bone repair. A key calcium phosphate ceramic mineral present in the human body. Hap is chemically and crystallographically equivalent to the mineral phase in bone. Hap has outstanding biological properties such as toxicity, lack of inflammatory response and absence of fibrous or immunological reactions. There has been a growing trend toward the development and use of biomaterials for repairing and restoration of damaged bone tissue and has been found to promote new bone formation when implanted in a skeletal defect. The aim of the present study is to know, phase composition, purity, crystallinity, crystallite size, lattice parameters, of different age groups of rat bone, lumber vertebra and hydroxyapatite. Inview, of this more fundamental radiation interactions from these samples, will provide additional data, with the use of synchrotron sources, with higher flux [1–5].

The functional properties of Hap strongly depend on their morphology, stoichiometric ratio, crystallinity and crystal size distribution.Some samples that are of special interest in archeometry, such as bone, scatter X-rays strongly and it is hard to obtain essential information conventional X-ray laboratory sources. This type ofdata can be obtained with detailed studies made with micro-focal optics at synchrotron sources.

The study of calcium phosphate-based ceramics has focused on mainly hydroxyapatite, [$Ca_{10}(PO_4)_6(OH)_2$],due to its increased bioactive potential shown in several investigations. It exhibits a hexagonal crystal system with space group P63/m and contains a total of 44 atoms per unit cell. The unit cell contains two crystallographic sites of Ca ions: columnar and screw-axis Ca. Columnar Ca ions are aligned parallel to the

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http://dx.doi.org/10.1016/j.msec.2016.11.136
0928-4931/

c-axis at approximate unit-cell heights of 0 and 1/2 and are linked by three shared O ions. Screw-axis Ca ions occur in two groups of three ions, with the Ca ions linked to a center OH group in the form of equilateral triangles. The triangles are orientated along the c-axis in the ab plane. The six screw-axis Ca ions are bonded to PO4 groups, and these PO4 groups can be further linked to other Ca ions in the unit cell. The small crystal plates of HAp [$Ca_{10}(PO_4)_6(OH)_2$] are typically 50 nm in length, around 25 nm wide and on average 3 nm thick. Stoichiometric HA, $Ca_{10}(PO_4)_6(OH)2$ in its commonest form, occurs as a hexagonally packed crystal [6–10].

The Ca/P ratio (10:6) of some of the calcium phosphates used in medical applications, for example, hydroxyapatite, is 1.67. Due to the close similarity between nanometer scale forms of HAp and the mineral phase found in the natural bone matrix. Recent studies have focused on understanding the structure of HAp for its inclusion in a new generation of novel composites. Bone mineral is constituted of biological hydroxyapatite crystals. In developing bone, the mineral crystal matures and the Ca/P ratio increases [11].

Synchrotron-based X-ray diffraction is a useful technique for determining crystal and molecular structures, to analyze the resulting diffraction data to determine the materials structure. To achieve this goal, several analysis techniques have been developed to resolve the diffraction data, since the publication of the original Rietveld method. With the advent of the Rietveld refinement technique, determining the crystalline structure from diffraction data has proven to be an indispensable tool for characterizing materials from X-ray diffraction data and as result, the technique is popular with crystallographers.

2. Experimental

Resonant and Diffraction beamline (P9) at Desy, Hamburg, Germany, is designed to operate the hard X-rays regime with energies ranging from 2.7 to 50 keV. Fig. 1 shows the experimental system used by the authors. X-rays with an energy of 15 keV ($\lambda = 0.82666\ A^0$) were selected from the bending magnet source using a silicon double-crystal monochromator. The experimental hutch is dedicated to resonant X-ray scattering and general diffraction experiments. The focus size is $150 \times 40\ \mu m^2$ and the energy resolution is <1.4 eV with Si (111) and for Si (311) it is <0.3 eV. The experimental hutch is equipped with a highly flexible and precise Psi-diffractometer with open Chi-circle. At the sample position, a simple goniometer mount, Displex cryostats or small sample environments, like ovens, can be mounted on the motorized xyz-translation stage. The detector arms consist of two translations separated by 25°. X-rays are detected using the area detector and mythen detector at an angle designated by the label 2θ, referenced to the direct incoming beam. The magnitude of the momentum transfer q is a function of 2θ, the scattering angle: $q = (4\pi/\lambda)\ \mathrm{Sin}\ (2\theta/2)$. The diffraction peaks presented in this work, varies upto 65 degrees ($0 < 2\theta < 65^0$), with a step of 0.02° at 5 s/step, with minimum sample tilts in the transverse direction. The data was converted to two-dimensional 2θ intensity data for the analysis of the samples.

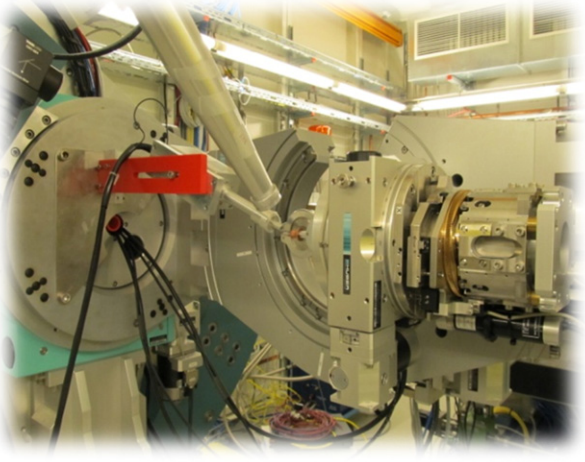

Fig. 1. Experimental system used by the author's at Desy: P9, diffraction beamline.

Table 1
Hydroxyapatite phantom dimensions.

Name	Sample	Dimensions	Hydroxyapatite (%)
BONEFIL 60 60	Phantom pellet		10 mm × 10 mm
BONEFIL 70 70	Phantom pellet		10 mm × 10 mm
BONECERAM 35–48	Phantom pellet		8 mm × 10 mm

3. Scanning electron microscopy

Researchers studied, the bone and associated morphology using SEM to magnify the features which will assist with identifying and classifying different spices. A high-resolution scanning electron microscope (HR-SEM, ULTRA Plus, Zeiss, Germany) was used for characterization of the crystal shape and morphology. To investigate the present samples, the SEM was operated at an accelerating voltage of 20 kV and current of 10 mA for imaging and the associated morphology. Samples were prepared for scanning electron microscopy (SEM) by mounting the dried samples on an aluminum stub covered in double-sided copper tape, then sputter coated with either Au/Pd or amorphous carbon.

4. Samples

A number of rat's bone and lumbar vertebra samples (10 × 10 mm; 10 × 8 mm) of different ages (8, 56 and 78 weeks) were prepared from Wistar rats, and these samples are used for XRD. All procedures were approved by the Animal Research Committee of the University.

5. Rietveld analysis

The raw data from the XRD scans were inputted into the FullProf program [12]. Rietveld analysis software program developed for crystallographic refinement. The HAp crystal model was built using information from the International Crystal Structure Database (ICSD). The dimensions of the hydroxyapatite are mentioned in Table1. The details

Table 2
Hydroxyapatite crystal structure parameters, refined by Rietveld analysis.

Phase data								
Space-group Cell					P63/m (176)—hexagonal $a = 9.4239$ Å, $c = 6.8841$ Å $c/a = 0.7305$, $Z = 1, \gamma = 120^0$ $V = 529.47$ Å^3, $\alpha = \beta = 90^0$			
Atom	Ox.	Wyck.	Site	S.O.F.	x/a	y/b	z/c	U [Å^2]
Ca1		4f	3…		1/3	2/3	0.00130	0.0037
Ca2		12i	1	0.33333	0.24620	0.99260	0.22220	0.0037
P1		12i	1	0.33333	0.39880	0.36910	0.22220	0.0048
O1		12i	1	0.33333	0.32600	0.48300	0.22220	0.0015
O2		12i	1	0.33333	0.58750	0.46530	0.22220	0.0015
O3		12i	1	0.33333	0.34070	0.25590	0.07040	0.0015
O4		4e	3…	0.5	0	0	0.20300	0.0015
H4		4e	3…	0.5	0	0	0.07300	0.0157