Research Note

X-ray Micro-Diffraction Analysis Revealed the Crystallite Size Variation in the Neighboring Regions of a Small Bone Mass

Toshiro Sakae¹⁾, Tetsuro Kono¹⁾, Hiroyuki Okada¹⁾, Hiroshi Nakada²⁾, Hidehito Ogawa³⁾,
Tsuneyuki Tsukioka³⁾ and Takashi Kaneda³⁾

¹⁾ Department of Histology, Nihon University School of Dentistry at Matsudo, Chiba, Japan

²⁾ Department of Removal Prosthodontics, Nihon University School of Dentistry at Matsudo, Chiba, Japan

³⁾ Department of Radiology, Nihon University School of Dentistry at Matsudo, Chiba, Japan

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Abstract: Applying a laboratory used X-ray micro-diffraction technique to analysis of human bone mineral, the variation of crystallite size in a small bone mass was clarified. The mandible cortical small masses were taken off by a bone trephine bar. The micro-XRD patterns showed the broadened peaks, but the full-width at half maximum (FWHM) of the 002 diffraction peak could be obtained. There were three characteristics on the FWHM values obtained in this study. First, the values showed a wide deviation range from 0.44 to 0.66 (2 θ°), indicating the crystallite length of 12.4 nm to 18.5 nm. Second, such a small FWHM value, 0.44, was not reported previously. And third, the variation of FWHM value was observed in the closely neighboring regions within one small bone mass separated within 1 mm only. These results suggested that the crystallites in bone may vary in size from to parts due to bone remodeling and histological textures.

Key words: Bone Mineral, Crystallite size, FWHM, X-ray diffraction, Peak Broadening

Introduction

As a part of the anatomical skeletal system in a human body, bone has been studied in a wide field from basic histology to applied material sciences, especially attracted the clinical attentions regarding to osteoporosis. Boskey *et al.*¹⁾ stated as that “the structural strength of bone is determined by bone quantity (i.e., mass, density, and size), geometric form, and bone quality, an elusive term that refers to internal architecture and material properties.”

Bone quality is a key to understand the bone fragility and fracture risk and also the adaptable conditions for dental implants²⁻⁶⁾. Among the techniques to assess the bone quality, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) are the two major applied techniques⁷⁻¹⁰⁾. Bone is composed of the organic and inorganic materials. The inorganic component of bone had been discussed for a long time because of the non-stoichiometric composition and diffuse X-ray diffraction patterns somewhat resembling to hydroxyapatite¹¹⁾.

Crystallite size in XRD study in practice measured as FWHM of a peak, because Scherrer Equation, $L = K \lambda / \beta \cos \theta$, was

Corresponding to: Dr. Toshiro Sakae, Department of Histology, Nihon University School of Dentistry at Matsudo, 2-870-1 Sakaecho-Nishi, Matsudo, Chiba, 271-8587 Japan; Phone & Fax: +81-47-360-9323; Email: sakae.toshiro@gmail.com

developed in 1918, to calculate the nano crystallite size (L) by XRD radiation of wavelength λ (nm) from measuring full width at half maximum of peaks (β) in radian located at any 2θ in the pattern. Although the Scherrer Equation is limited by the uncertainties in K, the crystallite shape factor, and β , the pure diffraction broadening¹²⁾, the equation is widely used for the ease to calculate¹³⁾. Monshi *et al.*¹⁴⁾ developed a modified Scherrer Equation to estimate more accurately nano-crystallite size and applied to the bone sample.

In this study, the well resolved peaks for the bone apatite could be obtained using a laboratory X-ray micro-diffraction instrument. Using this micro-diffraction technique, variation in the FWHM value was clearly shown from part to part in a bone sample. It was hoped that the more accurate crystallite size of bone mineral could be obtained than before. However, it was discussed why the results in this study still did not show the real crystallite size values, concerning the cause of XRD broadening of bone sample.

Materials and Methods

Bone Small Mass

The bone samples used in this study were from the patients who required a dental implant treatment with agreement for this study. This study was performed in accordance with the regulations

Figure 1. (Top) An extracted bone small mass showing the numbered colored point. (Bottom) A PSPC- X-ray micro-diffraction instrument used in this study showing the setting of the bone sample at the center of the curved PSPC detector.

of the Ethics Committee of Nihon University School of Dentistry at Matsudo (EC16-027).

Small masses of mandible cortical bone were taken off using a bone trephine bar with 3.8 mm bore diameter. These bone samples were kept in physiological saline solution. These bone samples were washed by distilled water and dried in air just before the following X-ray micro-diffraction experiment. Several points were marked by colored points for the ease of positioning in the X-ray micro-diffraction experiment (Fig. 1). The points to study were separated within 1 mm apart. Among the number of bone samples still being studied only two extreme examples were shown in this report, because it was necessary and sufficient number to show the availability of the X-ray micro-diffraction technique.

X-ray Micro-Diffraction (Micro-XRD)

A micro-diffraction instrument (RINT-2500, RIGAKU, Co. Ltd.) equipped with a curved position sensitive proportional counter (PSPC) was used in this study. The curved PSPC cached the diffracted X-ray in the range from 3 to 160 degree (2θ) all together (Fig.1). The experimental conditions were as follows; X-ray rotary target: Cu, accelerating voltage: 50 kV, current: 300 mA, monochromator: graphite, incident X-ray beam diameter: 100 μm , sample rotation axes: χ , ω , ϕ , counting duration: 2 hours.

The obtained X-ray diffraction data was analyzed with JADE (MDI, Materials Data Inc.). For comparison, the Powder

Figure 2. (Top) PSPC-micro-XRD pattern of a human mandible cortex bone, and (bottom) the mineral hydroxyapatite powder XRD pattern (RRUFF ID: R060180.1; Lafuente et al., 2015). The ordinate axis is the XRD intensity ($\times 1000$, cps). The broadened peaks of the bone XRD subsumed the corresponding hydroxyapatite peaks. The intensified baseline of the bone sample attributed to the reflections of the organic matters in the bone sample and from the circumstances because of the high energy X-ray used.

Diffraction File (ICDD, International Centre for Diffraction Data) for hydroxyapatite (PDF#09-0432) was used. The value of FWHM for the 002 diffraction peak at about 25.88° (2θ) was calculated using JADE.

Results

The obtained micro-XRD patterns in this study were as a whole almost the same to the micro-XRD pattern shown in Fig. 2. The main three peaks of the mineral hydroxyapatite, 31.80° , 32.22° and 32.93° (2θ , RRUFF ID:R060180.1 in Lafuente et al.¹⁵⁾ subsumed into one broaden peak in the bone micro-XRD pattern, and so forth on the other broadened peaks of the bone sample. These broadened peaks of the bone XRD patterns were similar to those reported by many investigators¹⁶⁻¹⁹⁾, and indicated that the bones were composed from the extremely fine grains.

Although almost all the micro-XRD peaks from the bone samples were broadened, each 002 diffraction peak at about 25.90° (2θ) in the micro-XRD patterns was isolated from the neighboring peaks, and therefore was able to measure the FWHM value for the 002 diffraction. Fig.3 showed the two bone samples having the different FWHM values. Table 1 listed the obtained FWHM values for the 002 diffraction peak together with the crystallite size along the c-axis calculated by the Scherrer Equation.

There were three characteristics on the FWHM values of bone

Figure 3. Two X-ray micro-diffraction patterns showing the difference in the peak broadening; (A) the FWHM value was 0.64, (B) 0.44, refer the data listed in Table 1. The lines indicated the positions and intensities of hydroxyapatite. In these XRD patterns showed the broaden peaks at about 20° (2θ) due to the organic matters

obtained in this study. First, the FWHM values showed a wide deviation range from 0.44 to 0.66 ($2\theta^\circ$); 0.0077 and 0.0115 in radian (Table 1). Second, such a small value, 0.44, in this study was not reported previously. And third, the variation of the FWHM values was observed in the closely neighbouring regions of one small bone mass. The examined neighboring points separated within 1 mm only.

The smaller FWHM value indicated the larger crystallite size. Applying the Scherrer Equation, with generally used constant $K=0.9$, the calculated crystallite size were in the range from 18.5 nm to 12.4 nm along the c-axis of apatite crystal (Table 1).

Discussion

Bone mineral substance is crystallized as nonstoichiometric carbonated apatite ionic crystals of small size and extended specific surface²⁰. About the initial and matured stage of the mineral phase of bone, there still be a subject of controversy. Real crystals lie somewhere between an ideal crystal state and an amorphous state. Presence of amorphous calcium phosphate (ACP) is in contention, whether to be^{21, 22}) or not to be^{23, 24}). The extent to which a solid is crystalline (crystallinity) has important effects on its chemical and physical properties²⁵.

In this XRD study, crystallinity was defined as the full-width at half maximum (FWHM) of a peak, and the value of FWHM represents the crystallite size and crystal lattice distortion²⁶. The

Table 1. List of the FWHM values for the two bone small masses.

Bone Sample	Position	FWHM		Crystallite size (nm)
		$2\theta^\circ$	radian	
A	1	0.64,	0.0112	12.7
	2	0.58,	0.0101	14.1
	3	0.56,	0.0098	14.5
	4	0.55,	0.0096	14.8
B	1	0.44,	0.0077	18.5
	2	0.66,	0.0115	12.4

crystallites in XRD study defined as the coherent diffraction regions, and generally the regions compiled up to a crystal observed under a transmission electron microscope (TEM). Thus the crystal sizes observed in TEM was often ten-times larger than those of XRD study²⁷.

Generally the XRD studies on bone cloud only show the broadened peaks such that the three hydroxyapatite main peaks at 31.773 , 32.196 , and 32.902° (2θ) usually form one broaden peak, unless using a high-energy nano-scaled beam XRD generated at synchrotron laboratory²⁸⁻³⁴). In this study, the obtained XRD patterns of the human bone samples showed the similar broadened peak pattern.

A number of XRD studies on bone crystallinity and crystallite size have been carried, and the differences in crystal size and the FWHM of bone were repeatedly observed^{20, 35-43}). As for the FWHM, this study disclosed three new findings: i) the FWHM values showed a wide deviation range, ii) a small FWHM value not reported previously, iii) the variation of the FWHM values in the closely neighbouring regions of one small bone mass. These results indicated that bone crystallite sizes were not in a small range but may vary in a wide range even in such a very close neighboring regions as within 1mm.

Two possible causes for the broadening of the bone XRD pattern of were as follows; i) the bone crystallite sizes being originally small enough, and ii) the broadened XRD pattern being superimposed of each a little bit sharper but slightly peak-shifted patterns due to the difference in chemical composition of each bone crystallites. Because the crystallites in bone had a variety in chemical composition^{11, 20}), and therefore the diffraction positions should vary.

Meanwhile the bone crystallite size changes with the age-related^{44, 45}) and with the type of bone tissue (corticalis, spongiosa)⁴⁶) were reported for animals, this was the first report for the crystallite size variation in the same bone small masses. The bone crystallites variations, including the crystallite size found in this study, may be attributed to the differences in the chemical properties induced by bone remodeling and also histological texture.

This study developed a new horizon of bone crystallites study using a laboratory X-ray micro-diffraction, meanwhile drew attention on handling and deciphering the obtained data. The

characteristics of the individual bone crystallites and of the assembled crystallites mass are to be studied.

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Conflict of Interest

The authors have declared that no COI exists.

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