Preferential orientation of biological apatite in normal and osteoporotic human vertebral trabeculae

S Miyabe, T Ishimoto and T Nakano

Division of Materials and Manufacturing Science, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

E-mail: nakano@mat.eng.osaka-u.ac.jp

Abstract. The preferential orientation of biological apatite (BAp) is a possible bone quality parameter for the comparison of the bone mechanical property. The preferential BAp orientation undergoes sensitive changes according to the change in the in vivo stress distribution, bone turnover rate etc., resulting in a variation of bone function. Osteoporosis is a metabolic bone disease characterized by reduced bone mass and deterioration of bone microstructure. The effect of osteoporosis on the preferential BAp orientation is however unknown. In this study, a microbeam-X-ray diffraction (µXRD) study was carried out on a trabecula extracted from osteoporotic and normal human vertebral bones and the degree of orientation for the BAp c-axis along its craniocaudal axis was analysed based on our previous report. A micro-computed tomography (µCT) measurement was also performed to analyze trabecular density and structure. In osteoporotic human vertebra, the trabecular number is markedly lower than that in normal vertebra. To sustain increased stress because of bone loss, the primary trabeculae, which are aligned parallel to the craniocaudal axis, tend to selectively remain while the secondary trabeculae, which are perpendicular to the craniocaudal axis, mostly disappear. Moreover, the primary trabecula from osteoporotic vertebra showed a significantly higher degree of BAp preferential orientation than the normal bone. This suggests that the remaining primary trabecula in osteoporotic vertebra is further reinforced by an increase in applied stress in vivo by enhancing the preferred BAp c-axis orientation along the trabecular direction.

1. Introduction
With the arrival of an aging society, osteoporosis is becoming a serious problem in the world. Osteoporosis is characterized by reduced bone mass and deterioration of bone microstructure, which increases the risk of fractures [1]. Vertebral bones are frequently evaluated in the diagnosis of osteoporosis and much attention has been paid to the trabecular bone in the vertebral bone because trabecular bones are believed to sustain 30-50% of vertebral strength [2]. Many studies have focused on the relationship between trabecular architecture and mechanical properties, while trabecular architecture has been reported to be a contributing factor to the mechanical properties of trabecular bone [3, 4]. Recently, our group reported that the preferential orientation of the biological apatite (BAp) c-axis as analyzed by microbeam X-ray diffraction (µXRD) is crucial in terms of bone reinforcement and is a possible candidate in the determination of bone quality parameters because of the crystallographic anisotropy of the hexagonal structure of the BAp crystallite [5]. However, little is understood about how osteoporosis changes the microstructural features and the related mechanical
function of bone. In this study, μXRD analysis was conducted on isolated osteoporotic and normal trabecular bones from human vertebral bones to investigate the pathological condition from a crystallographic point of view, in addition to the number and structure of the trabeculae.

2. Materials and Methods

Fourth lumbar vertebrae were extracted from male (69 year old) and female (86 year old) cadavers. Figure 1 shows soft X-ray photographs of the two specimens. Radiopacity of the male vertebra (a) is obviously higher than that of the female bone (b). According to the criteria for the diagnosis of osteoporosis as based on a two-dimensional X-ray projection and shown in Figure 1, the female and male vertebra were judged as osteoporotic and normal, respectively. These bone specimens were fixed in a 10% formalin neutral buffered solution to avoid infections and prevent denaturation of the organic matrix.

The three-dimensional trabecular structure was reconstructed by micro-computed tomography (μCT) (SMX-100CT-SV; Shimadzu). The volume fraction of trabecular bone (BV/TV, %) was calculated in a cube of 10 mm × 10 mm × 10 mm at the central part of the fourth lumbar vertebra by Tri 3D-BON software (Ratoc). Based on the 3D image, the rod-like primary trabeculae aligned within 5 degrees from the craniocaudal axis were removed from the central part of the fourth lumbar vertebral body to analyze BAp orientation (N=3).

Two-dimensional quantitative analysis of preferential BAp orientation was performed by a microbeam X-ray diffractometer system (D8 Discover with GADDS, Bruker AXS) equipped with a two-dimensional position sensitive proportional X-ray counter (2D PSPC) (Hi-STAR, Bruker AXS) using a transmission optical system. Cu-Kα radiation was generated at a tube voltage of 45 kV and a tube current of 110 mA and the incident beam was focused onto a beam spot of 20 μm in diameter by a mono capillary collimator. The degree of BAp orientation was evaluated as the integrated intensity ratio of the (002) diffraction peak to the (310) peak. The two peaks were measured separately based on their Bragg’s angles. Hydroxyapatite powder (NIST calcium hydroxyapatite #2910) with random orientation shows 0.7 of the intensity ratio in this system. Detail conditions for μXRD measurements were reported in our previous paper [6].

Data is expressed as the mean ± standard deviation (SD). Statistical analysis was performed using the student’s t-test. A P-value of <0.05 was considered to be significant.

3. Results and Discussion

Figure 2 shows μCT images of the central part of human fourth lumbar vertebral bodies. There is abundant trabecular volume and a three-dimensional trabecular network in the normal vertebra. In the osteoporotic vertebra, the trabecular number was markedly less, and the trabecular network was lost. The bone volume fractions (BV/TV) of the normal and the osteoporotic vertebrae are 16.3% and 5.5%, respectively. This difference in the bone volume between the osteoporotic and normal vertebrae suggests that in vivo stress applied to individual trabecula is greater in the osteoporotic bone than that in the normal bone. To sustain increased in vivo stress due to the loss of trabeculae, the primary trabeculae parallel to the craniocaudal axis tend to selectively remain while the secondary trabeculae perpendicular to the craniocaudal axis predominantly disappear.
Primary trabeculae were removed from the central part of the vertebra and μXRD measurements were conducted on them to analyze the BAp orientation in each trabecula. Figure 3 shows a typical isolated trabecula and a two-dimensional diagram of the degree of BAp orientation along all axes within a plane including trabecular direction. The higher value in the integrated intensity ratio of (002)/(310) indicates a more significant BAp c-axis orientation. A homogeneous distribution of the BAp c-axis within the plane results in a concentric diagram. Two-dimensional BAp distribution in the trabecula obviously indicates that the BAp c-axis distribution is highly anisotropic. The direction showing the maximum degree of BAp orientation corresponds precisely to the trabecular direction and the minimum BAp orientation is found in a direction perpendicular to the trabecular direction. Thus rod-like trabeculae have a uniaxial preferred orientation of the BAp c-axis, i.e. fibre-texture, along the trabecular direction. The maximum degree of uniaxial BAp c-axis orientation appears along the direction of the trabecular axis and is suitable to characterize the fibre-like BAp texture in the normal and osteoporotic trabeculae.

Figure 4 shows a comparison of the BAp preferred orientation between the normal and the osteoporotic trabeculae. The degree of BAp preferential orientation of the trabecula from the osteoporotic vertebra is significantly higher than that from the normal vertebra. It has been reported that in vivo stress is one of the dominant controlling factors of BAp orientation [5]. Figure 5 shows the correlation between the integrated intensity ratio of (002)/(310) and a type of in vivo stress component, which is represented by the reciprocal value of the trabecular volume fraction BV/TV. The degree of BAp orientation has a positive correlation to the in vivo stress, which indicates that even under the osteoporotic condition the trabecular bone can develop an anisotropic crystal texture and sustain increased stress cranio-caudally when applied in vivo and based on the functional adaptation mechanism of bone.

Bone functional adaptation is known to be expressed as the change in bone mass and bone mineral density when the applied stress distribution changes [7]. We conclude that if a decrease in bone mass is inevitable because of metabolic bone diseases like osteoporosis a functional adaptation can be
achieved by changing the degree of BAp orientation. Investigating the BAp preferential alignment mechanism is important for the understanding of changes in the mechanical function of bones when they are subjected diseases such as osteoporosis.

4. Conclusions
The crystal orientation of BAp in osteoporotic and normal human vertebral trabeculae was investigated by means of the microbeam X-ray diffraction technique and the following conclusions are made:
1. The preferred BAp c-axis orientation was increased significantly in the osteoporotic trabecula compared with that in the normal trabecula.
2. In the osteoporotic trabecula, increased in vivo stress, due to bone loss, enhances the degree of BAp preferred orientation in the trabecular direction and is based on the functional adaptation of bone.

Acknowledgements
This study was supported by the Priority Assistance for the Formation of Worldwide Renowned Centres of Research - The Global COE Program (Project: Centre of Excellence for Advanced Structural and Functional Materials Design) and a Grant-in-Aid for Scientific Research and Development from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. The work was partly supported by a Grant-in-Aid for JSPS Fellows (20 799). The authors would like to thank Prof. Y Nakajima and Prof. H Kiyama, Osaka City University for providing the bone specimen.

References
[1] 1991 Am. J. Med. 90 107
[2] Mazess R B 1990 Calcif. Tissue Int. 47 191
[3] Parfitt A M 1987 Am. J. Med. 82 68
[4] Ito M, Nishida A, Koga A, Ikeda S, Shiraishi A, Hayashi K and Nakamura T 2002 Bone 31 351
[5] Nakano T, Kaibara K, Tabata Y, Nagata N, Enomoto S and Umakoshi Y 2002 Bone 31 479
[6] Miyabe S, Nakano T, Ishimoto T, Takano N, Adachi T, Iwaki H, Kobayashi A, Takaoka K and Umakoshi Y 2007 Mater. Trans. 48 343
[7] Umemura Y, Ishiko T, Yamauchi T, Kurono M, Mashiko S 1997 J. Bone Miner. Res. 12 1480