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Texture of Biological Apatite Crystallites and the Related Mechanical Function in Regenerated and Pathological Hard Tissues

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Abstract: Preferential alignment of biological apatite (BAp) c-axis and the related collagen (Col) fibril was proved to be a dominant parameter showing bone quality for understanding nano-scale microstructure and the related mechanical function in addition to bone mineral density (BMD). We clarified the correlations between *in vivo* stress distribution and the BAp/Col alignment and between the BAp/Col alignment and mechanical function, especially Young's modulus, in original intact and regenerative hard tissues. The mutual relationships are predicted to be closely related to the function of osteocyte which can sense the surrounding stress field. The BAp orientation was finally concluded to be one of the most important indices to evaluate *in vivo* stress distribution, nano-scale microstructure and the related mechanical function, regenerative process of the regenerated bone and progress of bone diseases.

Key words: biological apatite (BAp), collagen (Col), preferential alignment, bone mineral density (BMD), Young's modulus, microbeam X-ray diffraction, nano-indentation, bone regeneration

Introduction

The bone mechanical function depends on both bone quantity and quality corresponding dominantly to bone mineral density (BMD: density of biological apatite) and the integrity of the internal architecture, respectively1). BMD is correlated with bone strength, but accounting only for 60-70% of the variance in ultimate strength of bone tissue2). Thus, new parameters representing the bone quality have been investigated so far.

Bone has a well organized microstructure in nano-scale level and is composed of mineral biological apatite (BAp) and collagen (Col) fibril, providing reinforcement and pliability, respectively3). Since BAp crystallizes in an anisotropic hexagonal lattice, mechanical properties of a BAp crystallite should depend on the crystal orientation. Moreover, the BAp c-axis accords with the extended direction of collagen fibrils4). Thus, preferential alignment of the BAp c-axis along the extended collagen fibrils in hard tissues must be closely related to the mechanical function of bone and is also utilized as a possible index for evaluating bone quality5,6).

In this article, we clarified correlations among *in vivo* external stress distribution, anisotropy of the BAp/Col alignment and the mechanical function evaluated by bone shape, BAp texture and Young's modulus, respectively, in the original intact, regenerated and pathological hard tissues.

Materials and Methods

Bone samples under the below conditions were prepared; (1) mature intact cortical bones such as a rabbit ulna, a rabbit skull bone, a monkey dentulous mandible with a tooth, a monkey vertebra (lumber 4: L4), (2) regenerated bones from the defected rabbit ulna model healed naturally. The specimens were immersed in a 10% formalin neutral-buffered solution to avoid denaturation of organic matrix. X-ray diffraction analysis for crystallographic approach to the constituent BAp crystallites was performed using the microbeam X-ray diffractometer system (M18XHF22-SR, Mac Science or D8 DISCOVER with GADDS, Bruker AXS). The incident beam was focused onto a beam spot 50µm or 100µm in diameter by a metal collimeter. The detailed conditions for the appropriate analysis of preferential alignment of the BAp c-axis should be referred in our previous paper5). BMD was measured by the peripheral quantitative computed tomography (pQCT) (XCT Research SA+; Stratec Medizintechnik, GmbH). Bone tissue was detected as CT values more than 267 mg/cm^3. Young's modulus was measured by nano-indentation tester (ENT-1100a, Elionix) at a loading/unloading strain rate of 400µN/s. Correlations among the analyzed parameters were assessed by Pearson's correlation coefficient. For statistical analysis, the Student's t-test was used and differences were considered to be significant at $P<0.05$.

Results and Discussion

Original intact bones exhibit unique texture of the BAp crystallites relating to the arrangement of Col fibrils5-7). Preferential alignment of anisotropic BAp crystallites in typical cortical bones, for example, changes depending on the bone shape and stress distribution *in vivo*. Figure 1 summarizes variations in the relative diffraction intensity ratio of the (002) diffraction peak to the (310) peak with different directions, A, B and C for the ulna, skull bone, dentulous mandible and lumber vertebra. It is clear that the preferential alignment of BAp c-axis corresponds to the *in vivo* stress distribution. The BAp c-axis tends to orientate along the loading stress direction in the original bones.

Figure 2 shows a change in correlations between BMD and the preferential alignment of BAp in the regenerated ulna as a function of regenerated period after the introduction of a 10 mm ulnar segmental defect accompanied with data in the original ulna.

Even after 20 weeks, recovery of BAp alignment depends strongly on the regenerated portion, which was insufficient to reach the original level, while BMD was almost improved to the original level. This means that BMD recovers prior to the improvement of BAp alignment and the related mechanical function in the regenerated tissue8-10). Thus, reloading on the regenerated portion

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caused by BMD restoration and subsequent filling of the defects are suggested to promote to produce the appropriate BAp/Col preferential alignment.

Young's modulus measured along the longitudinal direction shows a strong correlation to the BAp c-axis alignment in regenerated portion at 20 weeks in spite of the similar BMDs. This means that the increase in the BAp alignment promotes to increase the stiffness in the same direction. Moreover, the degree of preferential alignment showed a strong correlation with the local stress component expressed by the reciprocal value of the ulnar cross sectional area[9]. Thus, reloading by filling the defect caused by the BMD recovery becomes a trigger to improve the preferential BAp alignment towards the original one.

In conclusion, *in vivo* stress distribution produces the preferential alignment of BAp/Col, resulting in the change in Young's modulus. Degree of anisotropy in macroscopic external stress field is diminished inside of bones due to the preferential alignment of BAp/Col and subsequent increase in Young's modulus. This may mean that osteocyte can sense three dimensional stress field, and prefers isotropic stress field to anisotropic stress field.

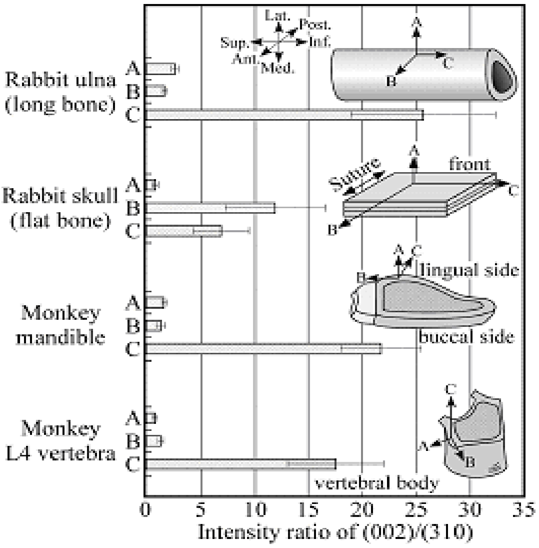

Fig. 1 Preferential alignment of the BAp c-axis in typical mature intact cortical bones.

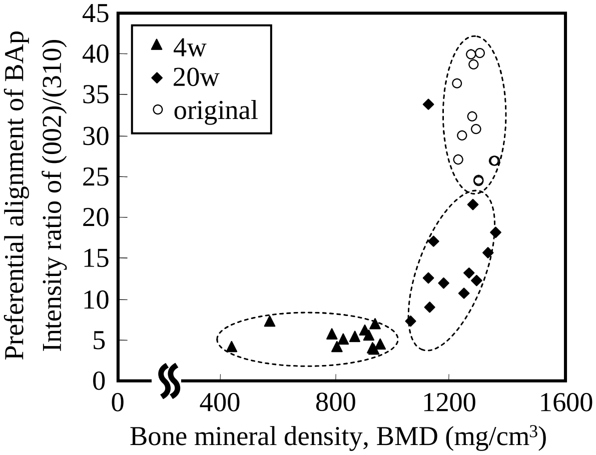

Fig. 2 Correlations between BMD and BAp c-axis alignment in regenerated ulnae along the longitudinal direction.

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