Adaptation of BAp crystal orientation to stress distribution in rat mandible during bone growth

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Abstract. Biological apatite (BAp) c-axis orientation strongly depends on stress distribution in vivo and tends to align along the principal stress direction in bones. Dentulous mandible is subjected to a complicated stress condition in vivo during chewing but few studies have been carried out on the BAp c-axis orientation; so the adaptation of BAp crystal orientation to stress distribution was examined in rat dentulous mandible during bone growth and mastication. Female SD rats 4 to 14 weeks old were prepared, and the bone mineral density (BMD) and BAp crystal orientation were analyzed in a cross-section of mandible across the first molar focusing on two positions: separated from and just under the tooth root on the same cross-section perpendicular to the mesiodistal axis. The degree of BAp orientation was analyzed by a microbeam X-ray diffractometer using Cu-Kα radiation equipped with a detector of curved one-dimensional PSPC and two-dimensional PSPC in the reflection and transmission optics, respectively. BMD quickly increased during bone growth up to 14 weeks, although it was independent of the position from the tooth root. In contrast, BAp crystal orientation strongly depended on the age and the position from the tooth root, even in the same cross-section and direction, especially along the mesiodistal and the biting axes. With increased biting stress during bone growth, the degree of BAp orientation increased along the mesiodistal axis in a position separated from the tooth root more than that near the tooth root. In contrast, BAp preferential alignment clearly appeared along the biting axis near the tooth root. We conclude that BAp orientation rather than BMD sensitively adapts to local stress distribution, especially from the chewing stress in vivo in the mandible.

1. Introduction
Bone is a well organized nano-structure dominantly composed of collagen fibers and biological apatite (BAp) crystals, as schematically described in Fig. 1 [1]. Since BAp crystal structure has an anisotropic hexagonal lattice [2], its crystal orientation and the related alignment of the collagen fibers, which are important parameters for controlling bone quality, are closely related to the mechanical properties of bone [3]. Bone quality denotes the important parameters except bone mineral density (BMD) and bone volume to explain the bone strength [4].

The preferential alignment of the BAp c-axis depends strongly on the bone portion [3, 5]. The long bone exhibits a one-dimensional alignment along its axis [6], but the rabbit skull has a two-dimensional alignment along the flat bone surface [5]. In the case of the mandible, the cortical portion separated from the tooth basically exhibits one-dimensional alignment along the mesiodistal axis [5, 7].
Figure 1. Schematic illustration of bone and bone microstructure composed of collagen fibers and biological apatite (BAp) crystals. BAp crystal shows an anisotropic hexagonal lattice.

Preferential BAp alignment along the mesiodistal direction also appears in the osteonal direction in human edentulous mandible [8]. Thus, BAp crystal orientation is closely related to stress distribution in vivo and the mechanical property of bone, but few studies have focused on BAp c-axis orientation in dentulous mandible in spite of its importance for understanding complicated stress distribution in vivo, the mandibular properties, the optimal design of dental implants, etc.

Figure 2. Variations in BAp crystal orientation in mature monkey mandible [5]. Degree of BAp c-axis corresponding to diffraction intensity ratio of (002)/(310) along the mesiodistal axis decreases near the tooth and that in the biting direction shows a local maximum on buccal side.
Based on three-dimensional analysis by microbeam X-ray diffraction, a unique change was found near the tooth crown, as shown in Fig. 2 [5]. The preferential degree of BAp crystal orientation quickly decreases in the mesiodistal axis when it approaches the tooth crown. On the buccal side, the local maximum in the BAp orientation degree corresponding to the diffracted intensity ratio of (002)/(310) especially appears in the biting direction. This suggests that local stress distribution in vivo is closely related to the BAp crystal orientation in spite of the complicated stress field in the mandible.

This study’s objective is to clarify the adaptation to the stress distribution during bone growth in the mandible by focusing on an increase in mastication force and/or number. In addition to bone shape and volume, two important parameters of bone were analyzed: BMD and BAp orientation. Finally, the changes in these two parameters were clarified to understand the variation in bone microstructure and to clarify the more important parameter during mandible growth.

2. Materials and Methods
Fifteen, 4-, 8-, and 14-week-old female Sprague-Dawley (SD) rats (Japan SLC Inc., Japan) (five per group) were used. Rats continuously grow due to their bone modeling and reach maturity around 20 weeks. The specimen cross-section contains the first molar tooth cut perpendicular to the lower border of the mandible (Fig. 3). The two positions, shown in Fig. 3(b) and labeled A and B, were analyzed because positions A and B are separated from and near the tooth root, respectively. Thus, position B must be directly influenced by the biting force.

A microbeam X-ray diffractometer (M18XHF22-SR, Mac Science; D8 Discover with GADDS, Bruker AXS) using Cu-Kα radiation was applied to analyze the preferential orientation of the BAp c-axis in two optics, reflection and transmission. Incident microbeams were focused into 50 and 100 μm φ in reflection and transmission optical systems, respectively. The diffracted beams were detected by curved one-dimensional position sensitive proportional counter (PSPC) and flat two-dimensional PSPC in the reflection and transmission optics, respectively [5, 9]. The transmission optical system was applied to the two-dimensional analysis of BAp crystal orientation using thin specimens polished to a thickness of about 150 μm. The degree of BAp c-axis orientation was evaluated by a diffracted intensity ratio of (002)/(310). See our previous article for details [9]. The bone morphology and volume were analyzed by micro CT, and BMD was evaluated by peripheral quantitative computed tomography (pQCT) (XCT Research SA+, Stratec Medizintechnik). Data were represented as a mean value ± standard deviation (SD). Statistical analysis was performed by one-way ANOVA and a two-tailed t-test. A P-value less than 0.05 was considered statistically significant.

3. Results and Discussion
It is common knowledge that the mandible grows under intermembranous ossification, and micro CT analysis showed that the bone area and the cortical bone thickness on the cross-section remarkably
increased by 14 weeks during bone growth. The alveolar bone portion of the mandible seems to grow rapidly with mastication.

Figure 4 shows that the BMD variations during bone growth in positions A and B separated from and near the tooth root, respectively. NS: not significant vs. position A.

Figure 5. Effect of mastication on degree of BAp c-axis orientation along the mesiodistal axis during bone growth. NS: not significant and *: P<0.05 vs. position A.
Figure 6. Effect of mastication on aligned degree of BAp c-axis orientation inside cross-section containing biting direction during bone growth. Integrated intensity ratio of (002)/(310) was adopted to evaluate preferential degree of BAp c-axis parallel to cross-section of mandible, as described in Fig. 3. NS: not significant and *: P<0.05 vs. position A.

Figure 6 shows the variations in the aligned degree of the BAp orientation inside the cross-section of the mandible containing the biting direction. The degree corresponds to the mastication effect as a function of age. In position B near the tooth root, the integrated intensity ratio roughly corresponds to the preferential aligned degree along the biting direction that increases quickly during bone growth up to 14 weeks. This good agreement with weight change may be closely related to the mass of the masticated food and may suggest that the aligned degree of the BAp crystal orientation along the biting direction corresponds to the mastication force and/or number due to the functional adaptation from local stress distribution in vivo.

A relationship among the biting, BAp preferential alignment, and cortical bone surface directions seems to exist. Detailed quantitative analysis will be described in a separate paper, but the preferred direction of the BAp c-axis seems to be located between the biting and bone surface directions, suggesting that the formation of BAp preferential alignment is strongly caused by biting and the morphology of the growing mandible.

4. Conclusions

The changes in two important parameters of BMD and BAp orientation were quantitatively evaluated in rat mandible during bone growth and the resultant increase in biting force and/or number; the following conclusions were reached:

(1) In the growth period up to 14 weeks, bone volume, BMD, and BAp orientation degree remarkably increase.

(2) The preferred BAp c-axis orientation is basically parallel to the mesiodistal direction to support the jaw, but mastication induces local alignment of the BAp c-axis roughly parallel to the biting direction. As a result, the BAp c-axis near the tooth root in position B exhibits two-dimensional alignment along the mesiodistal axis and biting direction as a function of age until 14 weeks.

(3) The change in the BAp crystal orientation is more sensitive to local stress distribution in vivo caused by mastication than that in BMD.
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