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Biological apatite crystallite alignment analysis of human maxillary molar region cortical bone with microbeam X-ray diffraction

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Abstract

The jaw bone is a specialized bone which in association with the masticatory muscles, moderates the occlusal forces transmitted to the jaw via teeth. It is suggested that there is a strong relationship between the structural characteristics of the jaw bone and occlusal force. Earlier studies involving bone density have shown clear differences between the properties of the maxilla and those of the mandible. It is reliable that assessment of the bone is also necessary to gain a better understanding of the material properties of the jaw bone, which maintains sufficient strength to withstand mechanical stress. In the present study, the author conducted a quantitative assessment of biological apatite (BAp) crystallite alignment, which is an indicator used in bone quality assessment, with the aim of clarifying the structural characteristics of the human maxilla.

Using dentulous maxillae from Japanese cadavers, interest, were measured the bone mineral density (BMD) and BAp crystallite alignment of first molar cortical bone, which was the area of interest. For measurement, the maxilla was classified into a total of four regions, consisting of cortical bone surrounding the alveolar process and cortical bone surrounding the root, on both the buccal and palatal sides, and each of these regions was assessed.

The results indicated no significant differences in BMD values based on site, but in the buccal cortical bone surrounding the root, strong alignment was observed in the vertical direction (Y-axis) in relation to the occlusal plane. Moreover, strong alignment was seen in the mesiodistal direction for palatal cortical bone compared to buccal cortical bone. At the same time, however, all of the measurement regions in the buccopalatal direction (Z-axis) showed low values for both buccal and palatal cortical bone.

Based on the results of the present study, it was confirmed that buccal cortical bone in the maxillary first molar cortical bone region has a strong mechanical characteristic in the Y-axis direction. This suggests that buccal cortical bone is more strongly affected by occlusal force, and it is surmised that it plays a significant role in transmitting stress generated primarily by the buccal root.

Keywords: Human maxilla, Biological apatite crystallite, Bone quality, Bone density, Microbeam X-ray diffraction

Introduction

The human jaw bone is a specialized bone that moderates occlusal forces generated as a result of the combined action of the masticatory and tongue muscles during function. Shimomoto *et al.* found that experimental animals with reduced occlusal function showed diminished growth of the alveolar bone and jaw bone.1) The magnitude of occlusal force was found to be positively correlated to the width and shape of the alveolar bone.2) In a study by Atwood *et al.*, morphological changes in the jaw bone with tooth loss were larger than the changes caused by aging.3) These findings suggest that there is a strong relationship between occlusal force and structural characteristics of the jaw bone.

The shapes of the maxilla and mandible differ greatly. Compared to the mandible, the maxilla has thinner cortical bone and cancellous trabecular bone.[4,5)] Park *et al.* compared the bone mineral density (BMD) of the maxilla and mandible based on Hounsfield unit (HU) values and found that the mandible had a higher BMD than the maxilla.[6)] Turkyilmaz *et al.* compared the BMD in the anterior tooth and molar regions in edentulous jaw bones and found the lowest BMD in the upper molar region,[7)] indicating that the maxilla and mandible have different characteristics. However, BMD is an indicator for quantitative bone assessment, and further information is needed to clarify the structural and qualitative properties of bone.

Bone quality has received much attention as a new bone assessment indicator in recent years.[8)] In particular, the alignment of biological apatite (BAp) crystallites, a bone quality indicator, responds markedly to local stress, and assessment of BAp alignment can therefore be used to identify the regions subjected to mechanical stress and the direction of the stress.[9-14)] Nakano *et al.* used a microbeam X-ray diffractometer to assess localized bone in the jaw bones of experimental animals, and reported that BAp crystals align preferentially in the direction of the masticatory force near the teeth.[9,14,15)] Moreover, Morioka *et al.* and Furuya *et al.* examined alignment of BAp crystallites in human mandibular cortical bone and found that the preferential alignment differed in the mandibular base area and the alveolar region where the teeth are located, proving that there is site specificity in the mandible.[16,17)] For the maxilla to exhibit high bone strength despite having a lower BMD than the mandible, the bone quality factors may play a major role. However, no studies have been conducted to examine the structural properties of the human maxilla based on bone quality assessment.

Author, therefore quantitatively assessed BMD and alignment of BAp crystallites in the cortical bone of the maxillary first molar region in human dentulous jaws in order to examine the effects of bone quality on the jaw bone and clarify the structural characteristics of the maxilla required to adapt to a dynamic environment.

Material and Methods

1. Samples

Samples were taken from six Japanese adult cadavers used for practical training (mean age 49.0 years) with no history of metabolic bone disease and with normal occlusion, from the collection at the Department of Anatomy, Tokyo Dental College. These cadavers possessed all of their dentition (both anteriorly and posteriorly).Maxillae were extracted from each cadaver (five male and one female) and severed at the median palatine suture to divide into right and left sides. The cadavers were fixed in 10% neutral buffered formalin solution before being dehydrated in ethanol for use in the study.

2. Micro-computed tomography(CT) scan

Micro-CT images of the samples (HMX-225 Actis4, Tesco Corporation, Japan) were acquired with the following imaging conditions: tube voltage, 140 kV; tube current, 93 µA; magnification, ×3; slice width, 50 µm; field of reconstruction, 60 mm; and matrix size, 512 × 512. Three-dimensional structure analysis software (TRI/3D-BON, Ratoc System Engineering Corporation, Tokyo, Japan) was used to create a 3D reconstruction and the internal structure was observed.

3. Measurement sites

The maxilla samples were embedded *in toto* in autopolymerizing resin. First, a diamond cutter (FineCUT, Heiwa Technica, Japan) was used to cut off the mesial and distal contact areas of the maxillary first molar region along the coronal plane. Next, the samples were sliced with a saw microtome with a blade thickness of 300 µm (SP1600, Leica, Germany) to obtain 150 µm-thick slices of the mesiobuccal root, palatal root, and distobuccal root on either side to yield a total of six slices per sample, centered around the root apex. The samples were polished with waterproof sandpaper (#400→#800→#1200) to eliminate the roughness from the sectioned surfaces.

For measurement, each sample was divided into the buccal area (Bu) and palatal area (Pa), with four measurement points set for each area (Fig. 1). The eight measurement points included two sites (I and II) in the cortical bone surrounding the buccal alveolar process (Region 1), two sites (III and IV) in the cortical bone surrounding the buccal root (Region 2), two sites (V and VI) in the cortical bone surrounding the palatal alveolar process (Region 3), and two sites (VII and VIII) in the cortical bone surrounding the palatal root (Region 4; Fig. 2).

4. Measurement of bone mineral density

BMD was measured with micro-CT imaging on the cut surface of the same areas as the slice samples. A CT value-bone mineral content calibration curve was prepared using phantoms (densities of 200 to 800 mg/cm^3) which measured φ6 × 1 mm and contained hydroxyapatite embedded in epoxy resin. BMD values were calculated from the calibration curve using 3D trabecular structure measurement software (TRI/3D-BON-BMD-PNTM2, RATOC System Engineering, Japan).

5. BAp crystallite alignment

Quantitative analysis of alignment of BAp crystallites was performed with the microbeam X-ray diffractometer with a reflection-based optical system using Cu-Kα beams and a transmission-based optical system using Mo-Kα beams (reflection system: RINT2500, Rigaku Corporation, Tokyo, Japan; transmission system: Rigaku R-AXIS Bone Quality, Rigaku Corporation, Tokyo, Japan). Samples were arranged with the mesiodistal direction along the X-axis, the vertical direction to the occlusal plane along the Y-axis, and the buccopalatal direction along the Z-axis (Fig. 3).For the reflection system, tube voltage was set at 40 kV and tube current at 200 mA. For the transmission system, tube voltage was set at 50 kV and tube current at 90 mA. The incident beam was focused on a minute 100 μm diameter spot for the reflection system and a 300 μm diameter spot for the transmission system using a collimator. Samples were first measured along the X-axis using the diffractometer of the optical reflection system. The diffracted x-ray beams were detected using a curved position-sensitive proportional counter. Samples were then measured along the Y- and Z-axes using the diffractometer of the transmission optical system. Measurement conditions were the same as those used by Nakano et al.9)

The transmission diffractometer produced diffraction rings on the imaging plate (IP) with diffraction lines (Fig. 4). X-ray diffraction data was recorded with Rigaku R-AXIS BQ software and evaluated by calculating the intensity ratio of the (002) and (310) diffraction peaks. The mean value of the measurements obtained from six fragments including the right and left sides of each sample at each of the eight measurement sites was taken as the measurement value.

6. Statistical analysis

For statistical analysis, Tukey's multiple comparison test was performed after calculating the means for two measurements in each region. A p value of less than 0.05 was considered statistically significant.

Results

1. Bone mineral density

The results of quantitative BMD assessment for each measurement region are shown in Figure 5. No significant differences were observed between any of the regions (1, 2, 3, or 4).

2. BAp alignment

The alignment of BAp crystallite in the X-axis direction in the mean for each measurement region is shown in Figure 6. The intensity ratio of HAp powder analysis used as a control was 1.21. BAp crystallite alignment in the X-axis direction was stronger in Regions 3 and 4 representing palatal cortical bone, than in Regions 1 and 2 representing buccal cortical bone.

Alignment of BAp crystallites along the Y- and Z-axes is shown in Figure 7 and 8, respectively, for each measurement region. The intensity ratio of HAp powder analysis used as a control was 0.60. Alignment was significantly stronger in Region 2 representing buccal cortical bone surrounding the root than in other regions.

In contrast, alignment was weak in all measurement regions along the Z-axis, and no significant differences were observed.

Discussion

1. BAp alignment

The results of the present study showed single-axis preferential alignment along the Y-axis in the buccal cortical bone which was particularly marked in cortical bone surrounding the buccal root. The reason for this may be that, in buccal cortical bone, occlusal force exerted via the teeth is transmitted to the cortical bone through the buccal root and alignment of BAp crystallites responds acutely to the resulting mechanical stress. Using simulation analysis, Cattaneo18) showed that stress resulting from occlusal force in the maxillary first molar region accumulates in the lower margin of the zygomatic process, indicating that the buccal area is affected by occlusal force in the maxilla. Peterson *et al.*19) measured the direction of greatest stiffness in maxillary cortical bone in human dentulous skull bones and found it to be parallel to the tooth axis in the molar buccal region. This is consistent with the direction of alignment of BAp crystallites observed in the present study. In the maxilla, the molar region has denser cancellous bone but thinner cortical bone than the anterior tooth region.19, 20) This suggests that bone quality factors may play a significant role in the maxillary molar region, and particularly in the buccal cortical bone, so that it can maintain the strength needed to withstand the occlusal force.

In palatal cortical bone, single-axis preferential alignment along the X-axis was observed. This may be because the palatal side of the maxilla shows long bone-like structure similar to the mandible, and this type of alignment may help retain the shape of the maxilla. Peterson *et al.* found the direction of greatest stiffness to be perpendicular to the tooth axis in palatal cortical bone in the first molar region.[19)] Moreover, measurement of the anisotropic elastic modulus in the maxilla and comparison with similar studies previously performed in the mandible showed similar anisotropy. The above findings indicate that the maxilla is a unique bone with different characteristics in the buccal and palatal cortical bones.

2. Relationship between BAp alignment and bone mineral density

No significant differences were observed in BMD value in any of the measurement areas. In contrast, there was a significant difference in BAp alignment along the Y-axis between the palatal cortical bone and the cortical bone surrounding the buccal root, in particular. This indicates that bone quality assessments such as alignment of BAp crystallites may be effective for assessing localized areas. Studies have shown that a decrease in mechanical stress from functional pressure does not affect BMD,[21-23)] and it may be necessary to assess bone quality in addition to BMD when quantitatively assessing the jaw bone.

3. Clinical implication

It was conjectured that the buccal cortical bone bears the occlusal force in the maxillary molar region. From this, it is suggested that stress generated from occlusion is transmitted to the cortical bone mostly around the buccal root. From a bone quality perspective, it is therefore possible that preservation of the buccal root may be inextricably linked to preservation of buccal cortical bone. These findings suggest that treatment to preserve the buccal root of the maxillary molar region and maintenance of a dynamic environment with dentures or implants in the buccal cortical bone after tooth loss are important for maintenance of bone quality.

Acknowledgements

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Figure and Legends

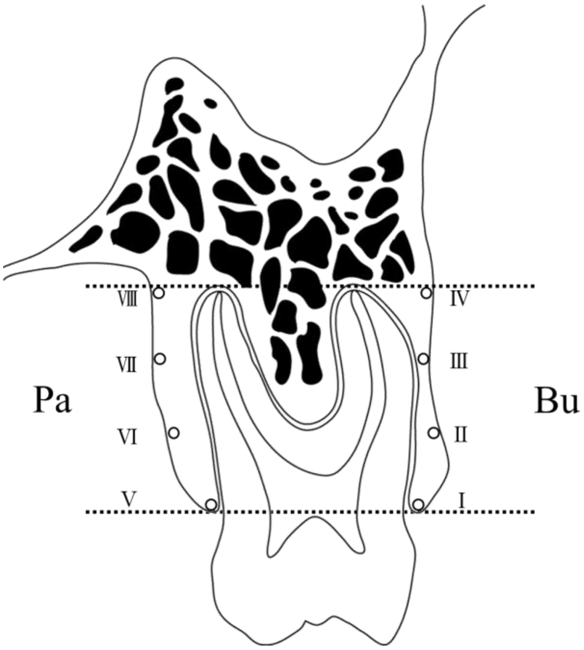


Fig. 1 Setting of the measurement points

The region of interest was the cortical bone on the apical region between the highest point on the alveolar process and each root.

Buccal area = Bu, palatal area = Pa

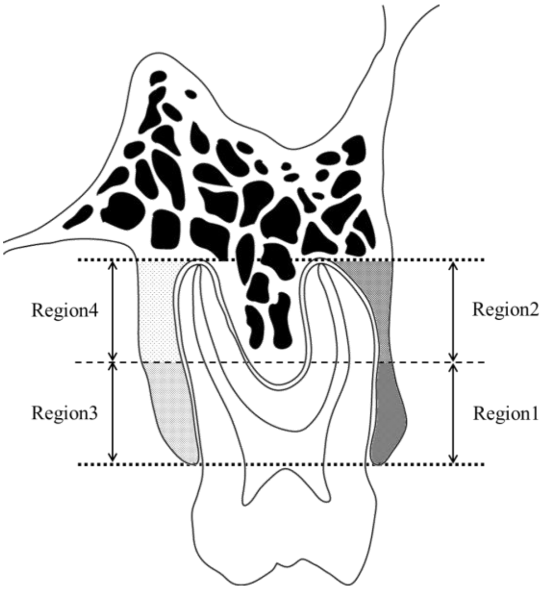


Fig. 2 Setting of the measurement regions

I/II: Cortical bone surrounding the buccal alveolar process (Region 1)

III/IV: Cortical bone surrounding the buccal root (Region 2)
V/VI: Cortical bone surrounding the palatal alveolar process (Region 3)
VII/VIII: Cortical bone surrounding the palatal root (Region 4)

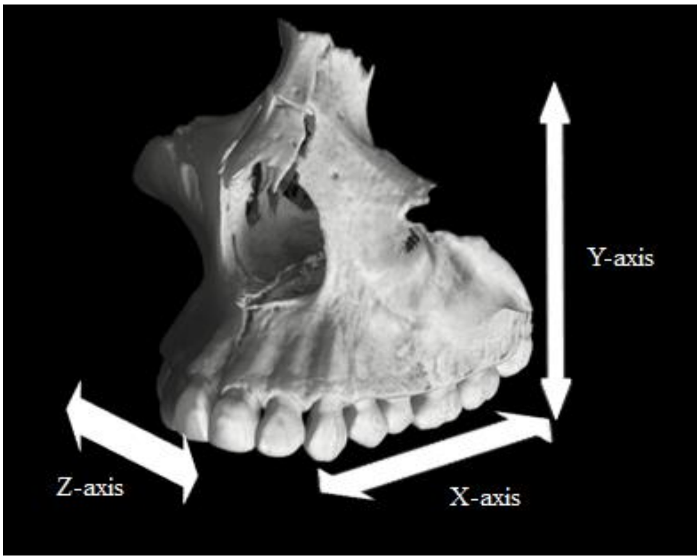
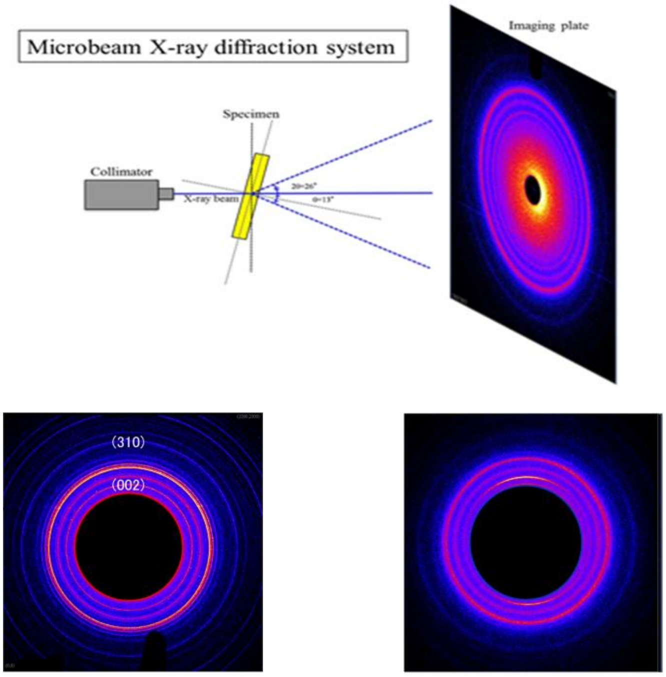


Fig. 3 Setting of the coordinate axes

Measurement sites were designated for the samples. The mesiodistal direction was placed along the X-axis, the vertical direction to the virtual occlusal plane along the Y-axis, and the buccopalatal direction along the Z-axis.



HAp BAp

Fig. 4 Microbeam X-ray diffiraction system and a measuring result example

The transmission diffractometer produced diffraction rings on the imaging plate (IP) with diffraction lines (Fig. 4). X-ray diffraction data was recorded with Rigaku R-AXIS BQ software and evaluated by calculating the intensity ratio of the (002) and (310) diffraction peaks.

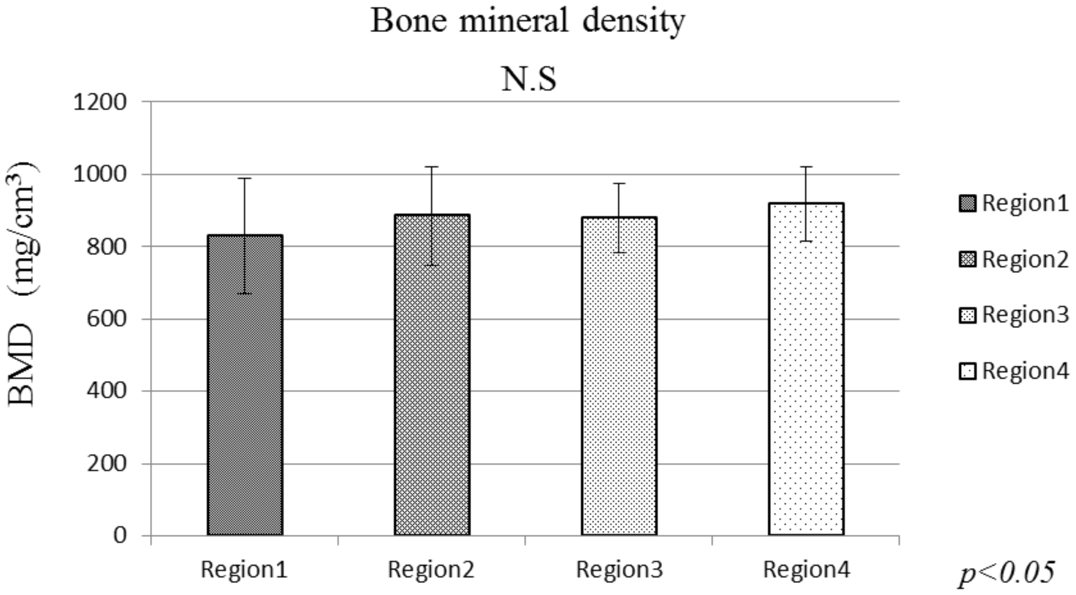


Fig. 5 Bone mass density values between regions 1, 2, 3, and 4

Vertical axis: Bone mass density value (mg/cm³), N.S: not significant

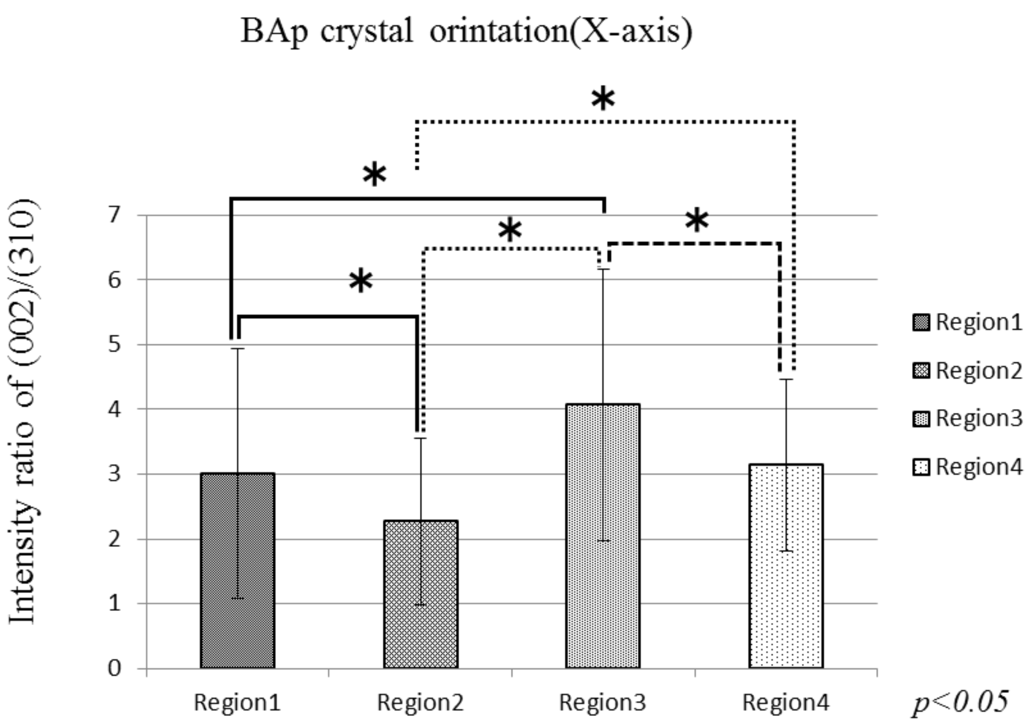


Fig. 6 Alignment of BAp crystallites along the X-axis (mesiodistal direction).
Comparison of each region. The vertical axis shows the diffraction intensity ratio calculated from the (002)/(310) peaks.

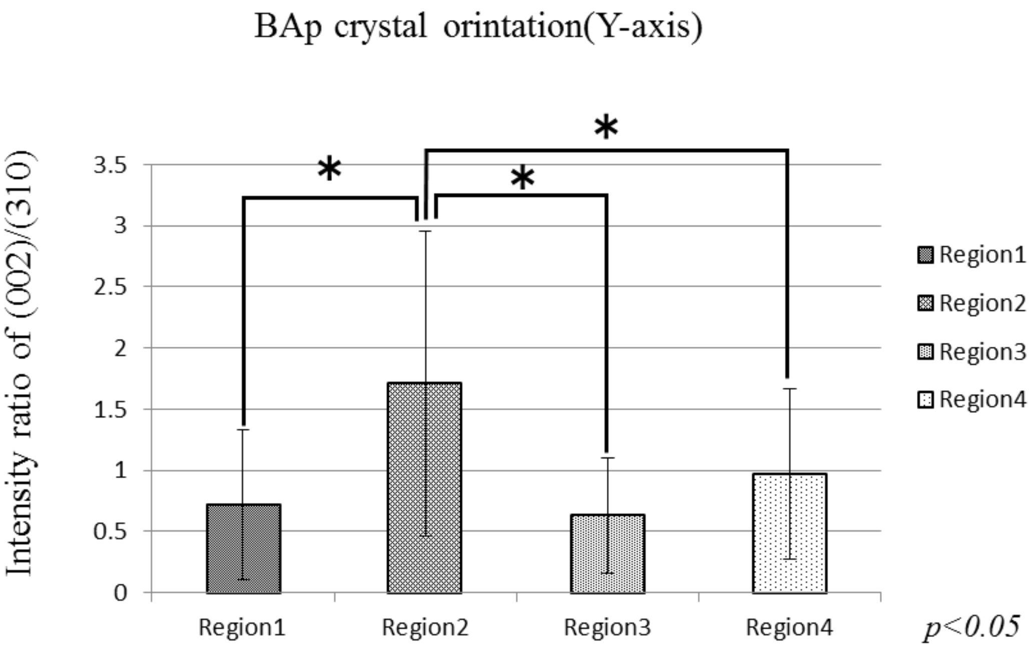


Fig. 7 Alignment of BAp crystallites along the Y-axis (vertical direction to the occlusal plane).

The horizontal axis shows the comparison of each region; whereas the vertical axis shows the diffraction intensity ratio calculated from the (002)/(310) peaks.

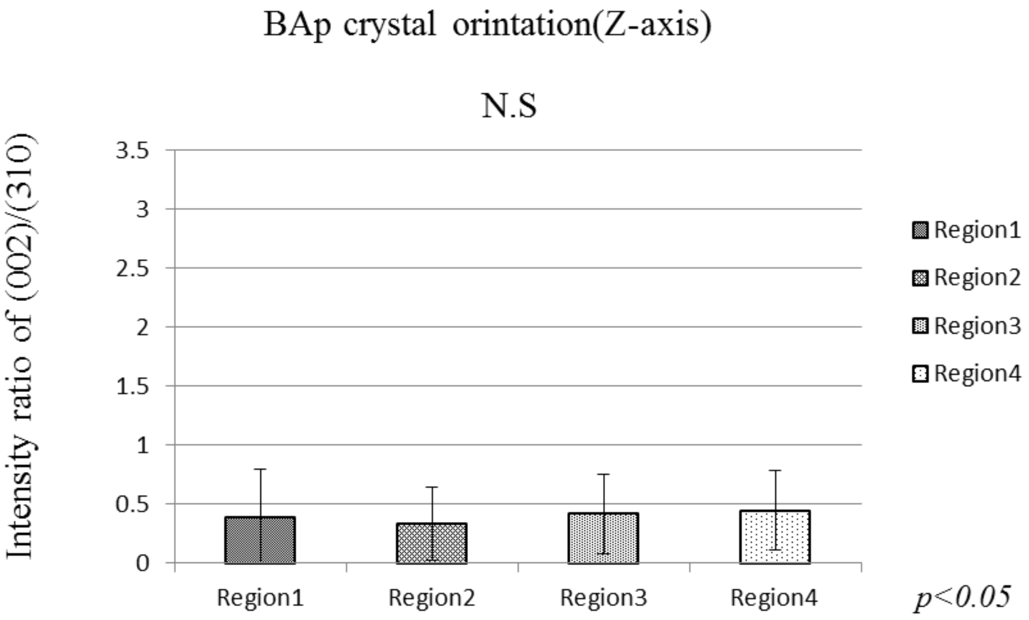


Fig. 8 Alignment of BAp crystallites along the Z-axis (buccolingual direction).

Comparison of each region with the vertical axis showing the diffraction intensity ratio calculated from the (002)/(310) peaks. NS: not significant