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Original

Alignment of Biological Apatite Crystallites in Posterior Cortical Bone of Human Edentulous Mandible

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Abstract: The mandible is a unique bone with a two-layered structure comprising the alveolar area that holds the teeth and the base of the mandible. When teeth are lost, the alveolar area is quickly resorbed, and the internal structure of the mandible changes greatly. Quantitative assessment of changes in the bone microarchitecture that occur with tooth loss is thus imperative. We therefore quantitatively assessed bone mineral density (BMD) and biological apatite (BAp) crystalline orientation in human edentulous mandibles and elucidated the structural characteristics of human edentulous mandibles. Japanese edentulous mandibles were divided into samples with a high and well-rounded alveolar area and thin cortical bone in the alveolar area (α-type), thick cortical bone (β-type), and those with a low and flat alveolar area (γ-type). BMD and BAp crystalline orientation were measured in the alveolar area and base of the mandible of the site corresponding to the first molar (the region of interest). Although BMD did not differ by site, comparisons of the different types revealed that BMD was high in the α-type and low in the β- and γ-types. BAp crystalline orientation in the alveolar area was observed in the vertical direction to the virtual occlusal plane (Y-axis) and buccalingual direction (Z-axis) in the α-type, whereas weak preferential orientation in the mesiodistal direction (X-axis) was observed in the β- and γ-types. BAp crystals in the base of the mandible showed uniaxial preferential alignment along the X-axis in all three sample types ($p < 0.05$). These findings demonstrate that most of the human edentulous mandible develops long bone-like characteristics with resorption of the alveolar area and that orientation in the alveolar area varies with morphological changes in the alveolar bone.

Key words: Human mandible, Biological apatite crystallite, Bone quality, Microbeam X-ray diffraction, Transmission method

Introduction

The human mandible is an extremely specialized bone with a masticatory function. It is subjected to functional pressure exerted by the teeth and is said to have unique structural properties. Because of this, loss of teeth drastically changes the external morphology of the mandible[1,2]. Individual differences are observed in mandibular bone resorption, and the extent of absorption also varies by site, even in the mandible. Changes are particularly notable in the alveolar area, where reductions are seen in alveolar height and width[3]. Studies analyzing the bone structure of dentulous and edentulous mandibles showed significant differences between the two in bone mineral density (BMD)[4], and studies are being carried out to explain the role of the load incurred via the teeth in changes in the internal structure of the mandible[5,6].

Meanwhile, bone assessment parameters have been expanded recently to include bone quality in addition to bone mass, and the former is garnering attention as an indicator for assessing bone strength that cannot be explained only by BMD[7]. Biological apatite (BAp) crystallites that are hexagonal and extremely anisotropic orient preferentially along the c-axis in the direction of collagen fibers, but this orientation responds strongly to the surrounding dynamic environment. Observation of that characteristic can clarify mechanical stress intensity and orientation in the crystallites. BAp crystalline orientation is therefore being studied as an important bone quality indicator[8-12].

Using neutron radiation to study human edentulous mandibles, Bacon *et al.* previously examined the relationship between attached muscles and crystallites and found that BAp crystallites in the

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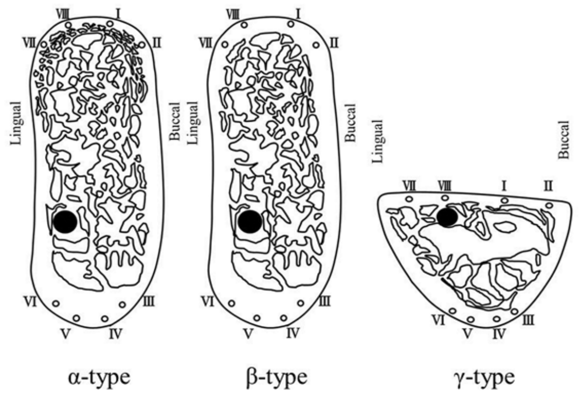

Figure 1 Structure of the coronal plane.
α-type: High, well-rounded alveolar area (0.80 mm or thinner).
β-type: High, well-rounded alveolar area (1.60 mm or thicker).
γ-type: Low, flat alveolar area .
I and II: sites on the buccal Al.
III and IV: sites on the buccal Ba.
V and VI: sites on the lingual Ba.
VII and VIII: sites on the lingual Al.

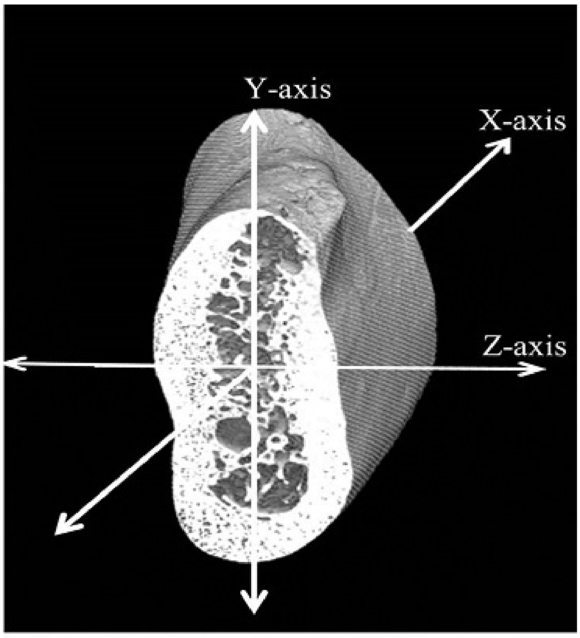

Figure 2 Setting of the coordinate axes.
Measurement sites were designated for the samples.
The mesiodistal direction was placed
along the X-axis, the vertical direction to the virtual occlusal plane along the Y-axis, and the buccalingual direction along the Z-axis.

mandible are oriented according to mechanical stress from the muscles[13]. More recently, a microbeam X-ray diffraction system developed by Nakano *et al.* has been used to measure localized BAp crystalline orientation. This system uses a collimator to focus X-ray beams from 10-100 ìm on a converging point, enabling quantitative evaluation of BAp crystalline orientation in microscopic regions of bone[8,14]. They then used this system in animal experiments to visualize a BAp crystalline orientation map in the mandible near the teeth[8,15,16].

Morioka *et al.* [17] and Furuya *et al.* [18] then uncovered differences in BAp crystalline orientation in the alveolar area and the base of the mandible in dentulous human mandibles that have more complex masticatory function than the animals that were previously studied. They reported that the base of the mandible exhibits long bone-like characteristics with the mandibular condyle constituting the head of the bone, whereas crystallites in the alveolar area are oriented in the direction of the masticatory force from mechanical stress exerted by the teeth. All studies on BAp crystalline orientation in cortical bone to date have only focused on dentulous mandibles.

In recent years, implant treatment has become an essential part of dental practice. Despite the critical role bone quality plays in determining the success of such treatment, no studies have yet been conducted to examine three-dimensional BAp crystalline orientation or the relationship between bone morphology and bone quality in human edentulous mandibles. Although the changes in human edentulous mandibles vary greatly among individuals, they are characterized by loss of the alveolar areas, resulting in changes in external morphology that are not generally seen in other bones.

We therefore assessed BMD and BAp crystalline orientation and the relationship between the two to elucidate differences in BMD and bone quality with the extent of alveolar bone resorption in the area corresponding to the first molar regions in human edentulous mandibles that are the main sites for implants.

Materials and Methods

Samples

Edentulous mandibles from the Tokyo Dental College Department of Anatomy collection were extracted from nine Japanese adult cadavers (mean age 79.3 years; seven men and two women) with no history of metabolic bone disease. The morphology of six samples was classified as a high and well-rounded alveolar area (α-type, β-type) and three samples as a low and flat alveolar area (γ-type). High and well-rounded samples were then further classified by cortical bone thickness (α-type: 0.80 mm or thinner, β-type: 1.60 mm or thicker). The posterior region from 9 mm from the center of the mental foramen was cut along the coronal plane perpendicular to the mandibular plane using a diamond cutter (Fine CUT, Heiwa Technica, Japan). The mandibles of cadavers were fixed in 10% formalin before dehydration in ethanol for use in the study. This study was approved by the Tokyo Dental College Ethics Committee (No. 326).

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, 75 μA;

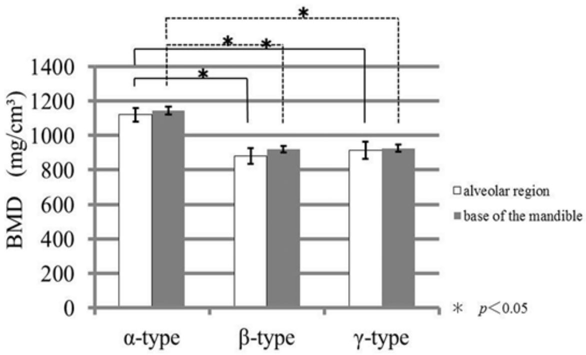

Figure 3 BMD values (mean ± SD) for Al (alveolar region) and Ba (base of the mandible).
Vertical axis: BMD value (mg/cm³) .
No site-based differences in BMD were observed between the Al and Ba of the human edentulous mandible in any of the three types. However, comparisons of the types revealed that BMD was higher in the α-type and lower in the β- and γ-types ($p < 0.05$).

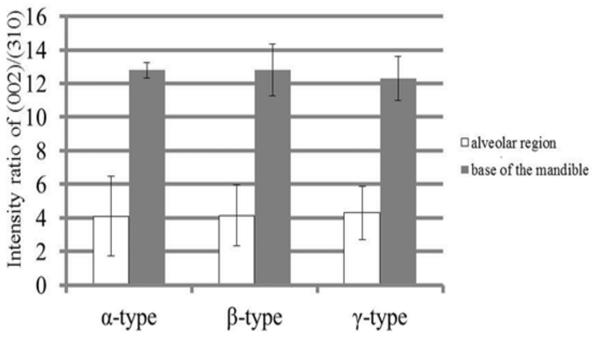

Figure 4 BAp crystalline orientation (mean ± SD) along the X-axis (mesiodistal direction).
Samples with a high and well-rounded alveolar area (α-type: about 0.80 mm or thinner; β-type: about 1.60 mm or thicker) and samples with a low and flat alveolar area (γ-type) were compared. Each sample was bisected into Al and Ba. The vertical axis shows the diffraction intensity ratio calculated from the (002)/(310) peaks.
BAp crystalline orientation along the X-axis in the α-, β-, and γ-type samples was stronger in Ba and weaker in Al in all three samples ($p < 0.05$ for both Ba and Al).

magnification, ×3; slice width, 50 μm; field of reconstruction, 60 mm; number of views, 1200; number of scans, 92; number of slices, 20; matrix size, 512 × 512. Three-dimensional structure analysis software (TRI/3D/BON, RATOC System Engineering, Tokyo, Japan) was used to create a 3D reconstruction, and the internal structure was observed.

Measurement sites

The samples were placed with the measurement side facing downwards, and autopolymerizing acrylic resin was injected and embedded from the top.

After embedding, the samples were sliced on the measurement side parallel to the coronal plane using a saw microtome with a blade width of 300 μm (SP1600, Leica, Wetzlar, Germany) to obtain one 200-ìm slice. The remaining pieces of embedded samples were polished with waterproof sandpaper (#400, #800 and #1200) to eliminate roughness from the sectioned surfaces.

The area corresponding to the alveolar region (Al) and the base of the mandible (Ba) were designated as the measurement areas, with four points set in each for measurement area, for a total of eight measurement sites. The measurement sites on the samples cut along the coronal plane were two sites on the buccal Al (I and II), two sites on the buccal Ba (III and IV), two sites on the lingual Ba (V and VI), and two sites on the lingual Al (VII and VIII; Fig. 1). Measurements of buccal Al site I and lingual Al site VIII were taken in sites 4 mm from the alveolar crest. The region under the cancellous bone in the Ba was bisected into the buccal side and the lingual side, which were used for measurement of the buccal and lingual Ba, respectively.

BMD measurement

BMD was measured with a peripheral quantitative CT (pQCT) bone densitometer (XCT Research SA+, Stratec Medizintech, GmbH, Pforzheim, Germany), with a voltage of 50.7 kV, current of 0.276 mA, and voxel size of 80 × 80 × 460 μm. Voxel data from each sample were scanned and read with Microsoft Excel and exported as an ASCII CSV file.

BMD of the cortical bone was measured in areas with a threshold of at least 690 mg/cm³.

BAp crystalline orientation

Quantitative analysis of BAp crystalline orientation was performed with a microbeam X-ray diffractor with a reflection-based optical system using Cu-Ká beams and a transmission-based optical system using Mo-Ká beams (reflection system: RINT2500, Rigaku Corporation, Tokyo, Japan; transmission system: Rigaku R-AXIS Bone Quality, Rigaku Corporation). Samples were arranged with the mesiodistal direction along the X-axis, the vertical direction to the virtual occlusal plane along the Y-axis, and the buccalingual direction along the Z-axis (Fig. 2). 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 50kV holds and tube current at 90 mA. The incident beam was focused on a minute 100ìm diameter spot using a collimator.

Samples were first measured along the X-axis using the diffractor of the reflection optical 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.*[8,13].

The transmission diffractometer produced diffraction rings on the imaging plate with diffraction lines. X-ray diffraction data were recorded with Rigaku R-AXIS BQ software and evaluated

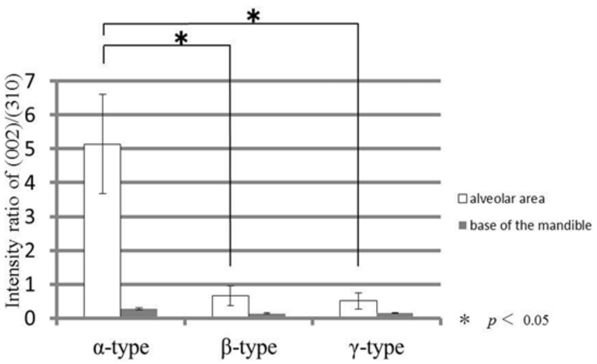

Figure 5 BAp crystalline orientation (mean ± SD) along the Y-axis (vertical direction to the virtual occlusal plane).
Samples with a high and well-rounded alveolar area (α-type: about 0.80 mm or thinner; β-type: about 1.60 mm or thicker) and samples with a low and flat alveolar area (γ-type) were compared. Each sample was bisected into Al and Ba.
The vertical axis shows the diffraction intensity ratio calculated from the (002)/(310) peaks.
In Al of the α-type where orientation along the X-axis was weakening, BAp crystalline orientation was high along the Y-axis. Moreover, comparisons of BAp crystalline orientation in the Y-axis directions in Al and Ba revealed significantly higher values in Al. In contrast, BAp crystalline orientation was weak along the Y-axes in both Al and the Ba in β- and γ-type samples.

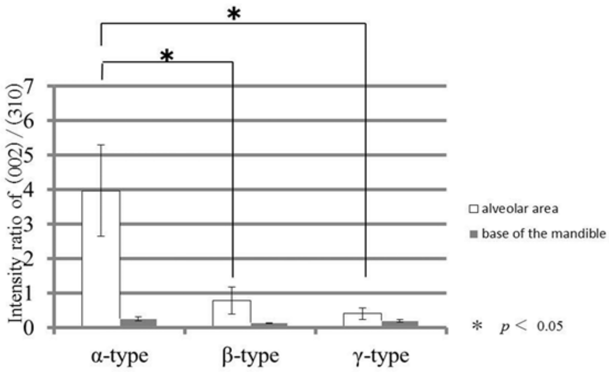

Figure 6 BAp crystalline orientation (mean ± SD) along the Z-axis (buccalingual direction).
Samples with a high and well-rounded alveolar area (α-type: about 0.80 mm or thinner; β-type: about 1.60 mm or thicker) and samples with a low and flat alveolar area (γ-type) were compared. Each sample was bisected into Al and the Ba.
The vertical axis shows the diffraction intensity ratio calculated from the (002)/(310) peaks.
In Al of the α-type where orientation along the X-axis was weakening, BAp crystalline orientation was high along the Z-axis. Moreover, comparisons of BAp crystalline orientation in the Z-axis directions in Al and Ba revealed significantly higher values in Al. In contrast, BAp crystalline orientation was weak along the Z-axes in both Al and Ba in β- and γ-type samples.

by calculating the intensity ratio of the (002) and (310) diffraction peaks. The means of three measurements in each of the eight sites were calculated and used as measurement values.

Statistical analysis

For statistical analysis, measurements along the X-, Y-, and Z-axes were divided into Al data and Ba data for the α-, β-, and γ-type samples, and the means of four points in Al and four points in Ba were compared with Tukey's multiple comparison tests. $p < 0.05$ was considered to be significant.

Results

BMD

The results of quantitative BMD evaluation for the means of four points in Al and four points in Ba with α-, β-, and γ-type measurements divided into Al data and Ba data are shown in Fig. 3. No site-based differences in BMD were observed between Al and Ba of the human edentulous mandible in any of the three types. However, comparisons of the types revealed that BMD was higher in the α-type and lower in the β- and γ-types ($p < 0.05$).

BAp orientation

Fig. 4 shows the BAp crystalline orientation along the X-axis measured with the reflection diffractometer for the means of four points in Al and four points in Ba with α-, β-, and γ-type measurements divided into Al data and Ba data. The intensity ratio of HAp powder used as a control was 1.53. BAp crystalline orientation along the X-axis in the α-, β-, and γ-type samples was stronger in Ba and weaker in Al in all three types of samples ($p < 0.05$ for both Ba and Al).

Fig. 5 shows BAp crystalline orientation along the Y plane measured with the transmission X-ray diffractometer for the means of four points in Al and four points in Ba with α-, β-, and γ-type measurements divided into Al data and Ba data. Fig. 6 shows the BAp crystalline orientation along the Z-axis measured the same way. The intensity ratio of HAp powder used as a control was 0.60 for both the Y- and Z-axes. In Al of the α-type where orientation along the X-axis was weakening, BAp crystalline orientation was high along the Y- and Z-axes. Moreover, comparisons of BAp crystalline orientation in the Y- and Z-axes in Al and Ba revealed significantly higher values in Al. In contrast, BAp crystalline orientation was weak along both the Y- and Z-axes in both Al and Ba in β- and γ-type samples.

Discussion

BAp orientation

In the present study, we found a uniaxial preferential alignment along the X-axis in Ba and along the Y- and Z-axes in Al in α-type samples. This observation suggests that the preferential orientation was in the longitudinal direction of the bone, because Ba has a horseshoe-shape and long bone-like structure with the mandibular condyle constituting the head of the bone. On the other hand, the orientation from the dentulous period may remain in Al, leading to preferential orientation along the Y- and Z-axes there. In addition, the extremely thin cortical bone in Al may have led to preferential orientation along the Y- and Z-axes to supplement those structural characteristics.

In β- and γ-type samples, preferential orientation along the X-axis was observed in both Al and Ba. Comparisons of BAp crystalline orientation in the X-axis directions in Al and Ba revealed significantly lower values in Al. These findings demonstrate that the human edentulous mandible shows a low response to mechanical stress with resorption of Al, resulting in most parts presenting with long bone-like characteristics.

The present study also found marked differences in the crystalline orientation of α- and β-types that were classified in the same group as mandibles with a high, well-rounded alveolar area. These differences may be due to a low response to mechanical stress with the thickening of cortical bone in edentulous mandibles.

This observation suggests that Nano-scale-level bone evaluation using BAp is therefore extremely useful. Further studies to determine the factors underlying differences between the α- and β-types may help clarify details concerning the changes in bone morphology of edentulous mandibles.

Relationship between BAp orientation and BMD

Comparing BMD in α-, β-, and γ-type mandibles revealed significant differences between the α-type and the other two types, with α-type samples exhibiting a higher BMD. However, BMD did not differ significantly between Al and Ba in any type of mandible. BMD is therefore useful for distinguishing the α-type from the β- and γ-types, which differ dramatically in bone mass, but cannot be used to make localized evaluations of each type of mandible. In contrast, BAp crystalline orientation is effective for qualitative evaluation by site. This result is consistent with findings on dentulous mandibles[17,18]. In the present study, we also found experimental evidence for the usefulness of BAp crystalline orientation for evaluating edentulous mandibles.

Clinical implication

In mandibles from which the teeth have been lost, BAp crystalline orientation becomes reset regardless of the extent of bone resorption, resulting in the loss of preferential orientation in the occlusal direction. When considering the clinical use of implants from the aspect of BAp crystalline orientation, it is recommended that implants should be placed while orientation along the Y- and Z-axes is still maintained.

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