Abstract: From the perspective of excellent biocompatibility and osteoconductivity of hydroxyapatite (HA), the frequency of use of HA implants has been steadily increasing in recent years. But the coating layer of certain commercialized HA implants may not be primarily composed of HA, and it may be assumed that the coating layers, contained some HA components, are automatically categorized to the HA implant. The characteristics of the implant to vital tissues are attributable to the properties of the surface components of the implant. Scanning electron microscope (SEM) observations and X-ray diffraction (XRD) analyses of the HA coating have been performed previously, but SEM has some difficulties in showing clear images of the HA crystal due to the influence of coating conditions etc., and the results obtained from XRD are not limited to the surface where contact with vital tissues, but also exhibit the inner components of the coating layer. Therefore, it might be assumed that reports limited to the top surface of the HA coating have still not been published. In this study, AFM observations, and XPS and micro-XRD analyses were performed on the top surface of five commercially available HA implants. AFM results showed that HA crystals were clearly observed on the surface of two samples. XPS and micro-XRD analyses exhibited that HA was detected in all the samples, but other substances including Ti, TiO$_2$, CaTiO$_3$, etc., were also confirmed in several samples. These results showed that HA was present on the top surface of all HA implants, and also indicated that all implants might exhibit some sort of biocompatibility and osteoconductivity due to their bioactive natures caused by HA.

Key words: Hydroxyapatite, Implant, AFM, XPS, Micro-XRD

Introduction

The base metal of implants is composed primarily of Titanium (Ti) or Ti alloy, and implant surfaces are generally classified into those composed of Ti or TiO$_2$, anatase crystal and those coated with hydroxyapatite (HA). Implants with Ti/TiO$_2$ surfaces (Ti implants) have long been recognized to show good biocompatibility due to chemical and physical stability. In contrast, HA-coated implants (HA implants) have indicated problems such as delamination, dissolution and infection, despite the fact they are considered to have good bioconductivity and osteoconductivity, resulting in delays in application to clinical practice.$^{1-3}$

However, in recent years, HA has been found to be the most stable calcium phosphate (Ca-P) that does not dissolve in body fluids, because body fluid is highly supersaturated with HA.$^{49}$ In addition, remarkable improvements in HA coating technology for base metals etc. have resulted in HA implants improving and getting high evaluations contrasting with the above-mentioned findings.$^{4-8}$ Furthermore, based on the bone reaction mode against HA in the in vivo environment, high temperature HA (HT-HA) is reported to be suitable for HA coating due to exhibiting osteoconductivity as a result of its bioactive/ non-bioresorbable nature. Low temperature HA (LT-HA) with low crystallinity produced at room temperature is reported to be suitable as a bone graft material due to bone substitution properties because of its bioactive/bioresorbable nature.$^{9-11,16-17}$ From the perspective of this current circumstances where no clear criteria for HA coating are available, some products have been referred to as HA implants simply because they contain HA in some form in their coating, but there are few cases that give the osteoconductivity arising from bioactivity/ non-bioresorbability as a feature, which is the greatest advantage of HA used in coatings. Furthermore, while it has been clarified that expression of osteointegration in Ti implants is due to HA and faint Ca-P compounds formed on the Ti/TiO$_2$ surface in vivo,$^{9-11,18}$ there are reports indicating an ambiguous theoretical basis for osteoconductivity and osteoconnectivity being expressed as a characteristic of an implant due to hydrophilicity/super-hydrophilicity. As a result, the only possible conclusion is that proper evaluations for implants in general have not been fully conducted. In addition, some products, which advertise the fact that they are HA implants, are primarily composed of substances other than HA, and there is a limit to the argument that HA content automatically categorizes a product as an HA implant. In addition, the characteristics of the implant with respect to the vital tissue are attributable to the properties of the substance constituting the surface, and the substances inside the implant should have little impact, but reports so far have mostly considered the entire coating layer including the inside. Scanning electron microscope (SEM) observations and x-ray diffraction (XRD) analyses for HA coating layer have been performed previously, but it is difficult to show clear images of the HA crystal by SEM due to the influence of coating conditions etc., and the results obtained from XRD are not limited to the surface where contact to
Furthermore, the surface roughness corresponding to each AFM image for clarifying three-dimensional discrimination in the AFM image. Due to large surface forms and the presence of unevenness etc., and the purpose of confirming the parts which were difficult to distinguish. Also, differential image Enhanced Color (EC) displays were made for the purpose of obtaining information limited to the surface roughness of the HA coating. Observations and analyses were performed on the flat area of the HA implant, AFM was the observation of the surface in a non-contact state. XPS was the surface analysis up to a depth of several nm, and micro-XRD was a surface layer analysis with a depth of about 6 μm or less.

The investigation of the arrangement/orientation and crystallinity of HA crystals in the surface of HA implants at the nano-level is an important task in elucidating the surface potential and surface properties of HA implants. It may also provide information essential to performing proper clinical evaluations of HA implants. Accordingly, in this study, atomic force microscope (AFM) observations and X-ray photoelectron spectroscopy (XPS) analysis were conducted on the HA implant surface in contact with the tissue that directly relates to biocompatibility and osteoconductivity. Additionally, micro X-ray diffraction (micro-XRD) analysis was conducted on the HA surface layer, and the results for the HA surfaces of each sample were compared.

### Materials and Methods

Implants that have been generally commercialized at present with surfaces indicated by the manufacturer to be HA coated were selected as samples (Table 1). The HT-HA (Taihei Chemical Industrial Co., Ltd., Osaka, Japan), used as a control, was prepared by heating Ca-P compounds (Ca/P molar ratio at 1.67) in the furnace at 1200°C for 6 hours. The HA coating layers were formed on the implants as follows: Implant-A: vacuum heat treatment after flame spraying of HA on the base metal surface, Implant-B: pressurized hydrothermal treatment using solution after plasma spraying of HA, Implant-C: thermal decomposition treatment, Implant-D: hydrothermal treatment after sputtering, Implant-E: Ca-P solution treatment. Two samples of each type were used, a flat area of the implant fixture was selected, and observation and analysis of each implant surface were performed quickly after opening the container (Fig. 1).

Observations by AFM were carried out in a completely non-contact state using a Park Systems (NX-20 type) atomic force microscope and cantilever (OMCL-AC160TS, Park Systems Japan, Tokyo, Japan), under conditions of 256 × 256 and 512 × 512 pixels and a scan rate of 0.2 to 1 Hz. Firstly, observations for the surface of each implant were carried out in the range of 20 × 10 μm, then observations were carried out while sequentially increasing the magnification ratio for parts where it was considered to be necessary. Observations were carried out up to the range of magnification whereby objects of about 100 nm can be identified and where the crystalline form of HA can be distinguished. Also, differential image Enhanced Color (EC) displays were made for the purpose of confirming the parts which were difficult to distinguish due to large surface forms and the presence of unevenness etc., and for clarifying three-dimensional discrimination in the AFM image. Furthermore, the surface roughness corresponding to each AFM image were given by line profile.

The PHI Quantera II manufactured by ULVAC-PHI, Inc. was used for analysis by XPS on the surface of the HA coating. XPS was conducted with the aim of obtaining reliable element information for a sample surface number of a few nm or less. After performing an overall qualitative analysis by survey spectrum (X-ray beam diameter: 100 mmf (100 W, 20 kV), analysis area: 1.4 mm × 0.1 mm, sample output: 50 kV, 100 mA, sample-counter distance: 20 cm, measurement angle: 45°, path energy: 280 eV), high resolution analysis was performed on Calcium (Ca), Phosphorous (P), Oxygen (O), and Ti, which are elements closely related to HA: Ca$_x$(PO$_4$)$_3$OH and the implant base metal, in a narrow spectrum (X-ray beam diameter: 100 mmf (100 W, 20 kV), analysis area: 1.4 mm × 0.1 mm, sample output: 45°, path energy: 140 eV). Measurement of the narrow spectrum was performed on the main spectra generated from the orbits of Ca 2p, P 2p, O 1s and Ti 2p, which showed the largest values for each element in the survey spectrum. Since measurement of H and He are impossible by XPS, measurement of O contained in the PO$_4$ and OH in HA was performed. In the analysis of each element, HT-HA, which was confirmed to have a high degree of crystallinity by powder X-ray diffraction, was used as a control.

In addition, it was judged that a surface analysis alone was insufficient considering the presence of surface roughness of the HA coating, so analysis by micro X-ray diffraction (micro-XRD) was conducted for the purpose of obtaining information limited to the surface layer. For the analysis of the surface layer of the HA coating layer, micro-XRD was conducted using an X-ray diffraction device D8 Discover with Vantec 2000 manufactured by Bruker AXS. The measurement conditions were X-ray source: Cu Kα (λ=15.4056 nm), output: 50 kV, 100 mA, sample-counter distance: 20 cm, measurement angle: 45°, path energy: 140 eV. Measurement of the narrow spectrum was performed on the main spectra generated from the orbits of Ca 2p, P 2p, O 1s and Ti 2p, which showed the largest values for each element in the survey spectrum. Since measurement of H and He are impossible by XPS, measurement of O contained in the PO$_4$ and OH in HA was performed. In the analysis of each element, HT-HA, which was confirmed to have a high degree of crystallinity by powder X-ray diffraction, was used as a control.
Observations were not possible by AFM. Therefore, such regions were where undulations were too large for detection by the cantilever and where identification by AFM was difficult, but there were also regions where particulate surface structure as a whole. Therefore, observation beyond the detection range of the cantilever was impossible, and sections represented as a plane in which no structure existed were observed in the line profile showing the form of the surface roughness in 20 × 10 μm on the right irrespective of the presence or absence of bulky or particulate surface structure as a whole. Therefore, observation beyond the detection range of the cantilever was impossible, and sections represented as a plane in which no structure existed were observed in the EC image.

**Implant-A**

In the image of 20 × 10 μm on the right of Fig. 4, it was confirmed that rounded bulky and block structures were formed on the entire surface. It was confirmed by EC that there were some structures in the region where observation was difficult by AFM. In 10 × 5 μm, cylindrical fine crystalline structures were formed on almost entire surface, and it was confirmed that the crystal was HA in 5 × 2.5 μm and 2 × 1 μm. In addition, it was confirmed that the HA crystals did not show constant regular arrangement or orientation. As was clear from the 1000 × 500 nm enlarged image on the left, the crystalline form of HA was clear with excellent crystallinity, and the arrangement/orientation was irregular. In 5 × 2.5 μm and 2 × 1 μm on the left, HA crystals were observed overall in the images of different sample surfaces in the same way as in the figures on the right, so it was judged that HA crystals were formed on the whole surface of B, and the coated state was excellent.

The line profile showing surface roughness at 20 × 10 μm on the right showed a relatively large undulation like roughness with fine...
Figure 3. AFM images of Implant-A:

There were regions on the surface where HA crystals were observed and those where they were not. The tendency for the crystals to regularly align and orientate on a plane from the c-axis was observed.
AFM images of Implant-B: HA crystals were observed on all regions of the surface. Regularity in the arrangement and the orientation of the crystals was not observed.
Figure 5. AFM images of Implant-C:
Sloping shape structures were generally observed, and recessed structures like crater shape were also found partially on the surface. Even when the image was enlarged, HA crystal or other crystal structure was not observed.
Figure 6. AFM images of Implant-D: Surface undulations were relatively large, but HA crystal or other crystal structure was not observed. The image supposed to be a base metal was partially observed.
Figure 7. AFM images of Implant E:
The region that appeared to be the crystal-like structure was observed, but clear HA crystals could not be confirmed.
roughness was confirmed, but undetectable region was not observed. It was confirmed that the distribution range of the roughness in the sample was about 5 to 8 μm in the state including the undulations.

**Implant-C**

In the 20 × 10 μm and 10 × 5 μm images on the right of Fig. 5, sloping shape structures were generally observed, and crater like recessed structures were also formed partially on the surface. Even when the image was enlarged to 5 × 2.5 μm and 2 × 1 μm, crystal or crystalline structure was not observed. This tendency was the same as 5 × 2.5 μm and 2 × 1 μm on the left for the different sample surfaces, and the crystal structure was not observed.

The line profile for 20 × 10 μm on the right showed relatively smooth undulations and slight roughness, and it was confirmed that the distribution range of roughness was within several μm.

**Implant-D**

In the 20 × 10 μm and 10 × 5 μm images on the right of Fig. 6, structures with comparatively clear shapes such as particulate, bulky and block shapes were generally formed on the surface, and it was confirmed that the irregularities and undulations on the surface were relatively large. This tendency was also similar to the 5 × 2.5 μm and 2 × 1 μm images, and although relatively smooth and sharp structures were observed, crystalline structures could not be observed. Also, as shown in 2 × 1 μm for the same sample on the left, the image supposed to be a base metal was also observed. In 5 × 2.5μm and 2 × 1μm on the left, findings similar to the right of Fig. 6 were observed for different sample surfaces, and no crystalline structure was observed.

The line profile for 20 × 10 μm on the right showed a relatively coarse. The line profile for 20 × 10 μm on the right of Fig.6 exhibited relatively clear recesses, and was confirmed that the distribution range of roughness was within around 5 μm.

**Implant-E**

In the 20 × 10 μm and 10 × 5 μm images on the right of Fig. 7, structures with indefinite but distinct forms such as particulate, bulky and block shapes were formed on the whole surface. This tendency was almost the same, even in the 5 × 2.5 μm and 2 × 1 μm images, and the region that appeared to be a crystal-like structure was also observed but clear HA crystals could not be confirmed. Those findings were similar to 5 × 2.5μm, 2 × 1μm on the left of Fig. 7 for different sample surfaces.

The line profile for 20 × 10 μm on the right showed the presence of obvious unevenness and it was confirmed that the distribution range of surface roughness was within around 5μm.

**XPS analysis**

The measurement was carried out keeping in mind that the analysis sample surface was mainly consisted of HA and Ca-P. Fig. 8 showed the qualitative analysis results by survey scanning spectrum, and Fig. 9 show high resolution analysis results by narrow scanning spectrum. Also, Table 2 shows ratios of the elements in the narrow spectrum. In the measurements, since photoelectron waveforms emitted from elements other than Ca, P, O, Carbon (C) and Ti were hardly observed, the graph showed the four elements excluding C, which was primarily originated from the atmosphere and contamination. As shown in the graphs of the survey spectrum in Fig. 8, according to the HA of the control, no spectrum was detected other than the spectra of Ca, P, and O which are main constituents of HA, and the spectrum for C taken in over time. Also, as shown in Table 2, there was no significant difference in the composition ratios of Ca, P, O and C of all the samples as compared with those of the control. The presence of Ca, P and O, which are the main components of HA, was confirmed from all the sample surfaces, but the spectrum of Ti was also observed depending on the sample.

The Ca 2p spectrum of HA in the narrow spectrum was the binding energy belonging to calcium phosphate (CaPO_{1-x}O_{2-x}■) attributed to Ca, which is the main constituent of HA, and the spectra of P 2p and O 1s were the binding energy belonging to phosphate (-PO_{1-x}O_{2-x}■). Also, as shown in the AFM line profile, the sample surface was not flat, but was rough and has undulations. Since roughness was clearly different among samples, and the uptake or adsorption of C into a sample occurred, analysis was carried out with a view to learning the relative tendencies with respect to the HA related elements on the sample surface.

**Implant-A**

In the analysis results by the survey spectrum, the presence of Ca, P and O, which are the main components of HA, was confirmed in the sample, (Fig. 8). High resolution analysis of Ca, P, O, by the narrow spectrum confirmed that the constituent ratio of each element was relatively low in comparison to the photoelectron intensity of the control HA (Fig. 9). This suggested that HA on the surface did not exist on the whole surface but other Ca-P might also exist in parts. As also shown in Table 2, Ti was not detected, but the detection of C was relatively high.

**Implant-B**

In both the survey spectrum and the narrow spectrum, almost the same waveform for elements related to HA as the control were exhibited, suggesting that the elements were mostly due to HA (Figs. 8 and 9). In particular, with the exception that P showed a slightly lower value than the control, it was confirmed that the content ratio of each element was quite close to the control from the fact that Ca and O have nearly the same waveform as HA. Also, as shown in Table 2, the Ca content of Implant-B was the highest among the samples, Ti was not detected at all and the content of C was low. It was confirmed that photoelectron intensities and waveforms of the element were quite similar to those of the control.

**Implant-C**

Although the clear presence of HA crystals was not confirmed by AFM, similar photoelectron intensities and waveforms for HA elements were shown in both the survey spectrum and the narrow spectrum, the values were relatively lower than the control (Figs. 8 and 9). It was highly likely that intensities and waveforms of HA-related elements were also detected from substances other than HA containing the elements, and although the presence of HA was clear, it was suggested that the surface coverage of HA was partial. In addition, a relatively clear waveform was detected for Ti, which was not detected in the control, and the binding energy value of the spectrum for it was

| Table 2. Quantitative results by narrow scanning spectrum: Constituent ratios of the element in the narrow spectrum are shown in atomic % |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Ca 2p | P 2p | O 1s | Ti 2p | C 1s |
| HA/Control     | 16.7   | 11.3 | 53.6 | 0.0   | 18.4 |
| Implant-A      | 16.6   | 10.5 | 50.8 | 0.0   | 22.1 |
| Implant-B      | 19.2   | 10.6 | 56.9 | 0.0   | 13.2 |
| Implant-C      | 17.3   | 11.2 | 56.5 | 2.1   | 12.9 |
| Implant-D      | 16.8   | 10.4 | 51.9 | 0.3   | 20.6 |
| Implant-E      | 16.2   | 10.5 | 56.7 | 0.0   | 16.7 |

(Atomic %)

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confirmed to be mainly amorphous TiO$_2$ through comparison with the standard measurement waveforms of Ti/Ti compounds (Fig. 9). The above results demonstrated the presence of TiO$_2$ on the surface. Also, as shown in Table 2, Implant-C showed the highest Ti content of the samples, but the C content was low.

**Implant-D**

Although the clear presence of HA crystals was not observed by
AFM, a comparatively similar waveforms for HA elements was shown in both the survey spectrum and the narrow spectrum, although the values were clearly lower than the control (Figs. 8 and 9). This results suggested that HA existed partially on the surface of Implant-D. In addition, the presence of Ti was confirmed though in small quantity, suggesting the possibility of TiO$_2$ having crystallinity from its binding energy value (Fig. 9). As shown in Table 2, the Ti content of Implant-D was low but the C content was relatively high.

**Implant-E**

No clear presence of HA crystals was observed by AFM observation, but the presence of Ca, P and O was confirmed by the survey spectrum analysis (Figs. 8 and 9). Analysis by the narrow spectrum confirmed that intensities and waveforms of HA-related elements were similar to those of the control, but those values were slightly lower than those of the control (Figs. 10 and 11). Also, the spectrum of Ti was not detected (Fig. 10 and Table 2). It was confirmed that the elemental composition and content of the surface are relatively close to the control.

**Micro-XRD analysis**

The results obtained from X-ray diffraction (XRD) for HT-HA powder, which was the same control as for XPS, and micro-XRD for each HA coating sample were shown in Fig 10. Identification of the detected substance was carried out by comparison with standard powder XRD data (Powder Diffraction File: PDF) for each substance.

**Implant-A**

Although the thickness of the HA coating layer was reported to be about 20 μm, Ti$_3$O (titanium oxide) was also detected in addition to the detection of a clear Ti diffraction pattern from the surface layer to about 6 μm. Ti on the surface was not detected by XPS, but the presence of Ti in the shallow part of coating layer was confirmed. Although detection of HA was observed, its intensity was relatively weak and it did not constitute the main phase of the surface layer of the coating. In addition, it was suggested that the peaks of the HA diffraction pattern were biased toward the orientation of crystal in comparison with those of the PDF and the control.

The above results revealed that HA was partially present in the surface layer, in the same way as the results of AFM and XPS for the surface. It was observed by AFM that the HA crystals tend to be arranged/oriented from the c-axis with respect to the surface, but the micro-XRD confirmed the fact that the orientation of the crystals was biased from a comparison between the diffraction pattern and PDF. Judging from all the results, it was confirmed that HA and Ti were the main constituents of the surface and the surface layer of Implant-A.

**Implant-B**

Although the HA coating layer was reported as 50 to 70 μm, Ti and Ti oxide from the surface layer was not detected. Almost all of the diffraction patterns had HA as the main phase, and their detection intensities and peaks were also quite similar to those of the PDF and the control.
control. In addition, faint CaCO$_3$ diffraction pattern was also detected, but most of it was buried in the main phase HA diffraction pattern and detection might not have been sufficient for identification. Since the peaks of each of the HA diffraction patterns were almost the same in comparison to those of PDF and the control, it was suggested that there was no bias in the arrangement and orientation of HA crystals.

Since the above results were almost the same as the results of AFM and XPS, it was confirmed that the surface and the surface layer of the coating were almost entirely composed of HA. Moreover, it was observed by AFM that the HA crystal was aligned in an omnidirectional arrangement/orientation with no bias, and this tendency was confirmed similarly by micro-XRD.

**Implant-C**

A diffraction pattern was detected with Ti and Ti$_2$O as the main phases, which were thought to be derived from the base metal, since the HA coating layer was reported to be relatively thin at 3 to 5 μm. In addition to the clear detection of TiO$_2$ (Rutile) produced by heating Ti at high temperature, a weak diffraction pattern of CaTiO$_3$ (Perovskite) was confirmed. Although the HA diffraction pattern was observed, the diffraction intensity was much weaker than that of Ti.

Clear HA crystals were not observed by AFM on the surface of the coating, but the presence of HA and TiO$_2$ on the surface was confirmed by XPS, and in the analysis of micro-XRD, Ti and Ti oxides were main constituents in surface layer. Above those results, the top surface of Implant-C was determined to be primarily composed of Ti, Ti compounds and HA.

**Implant-D**

A clear HA diffraction pattern was detected in addition to a diffraction pattern with Ti as the main phase, which might originate from the base metal since the HA coating layer was reported to be extremely thin at 1 to 2 μm. Since peaks could be observed overall in the HA diffraction pattern, it was assessed that there was no bias in the crystal arrangement.

HA crystals were not observed by AFM on the surface, but the slight presence of crystalline TiO$_2$ was shown together with HA in XPS, and in the analysis of micro-XRD in the surface layer, Ti and HA were confirmed to be the main constituents. That is, the top surface of Implant-D was shown to be mainly composed of HA and Ti.

**Implant-E**

Since the thickness of the HA coating layer was considered to be several tens of μm, detection of substances derived from the base metal was not observed, and a diffraction pattern with HA as the main phase was detected.

Almost no HA was observed on the surface by AFM, but XPS showed that HA was clearly present, so it was confirmed that the coating surface layer were mainly comprised of HA.

**Discussion**

Due to its excellent biocompatibility, osteoconductivity, and physical and chemical properties, HA has been widely used as implant coatings and bone grafting materials. It has also been used in clinical applications such as dentin hypersensitivity and remineralization of teeth. HA is often considered to generally be uniform and to consistently exhibit the same properties, and the fact it exhibits different properties according to different production temperatures is not well recognized. HA is classified as HT-HA and LT-HA according to the difference in production environment. The HA used for coating is mainly HT-HA. HT-HA is produced by blending at the raw material Ca/P (calcium/phosphorus) ratio of 1.67. It is then fired at the high temperature of 1000 to 1400°C and quenched. HT-HA has an extremely high degree of crystallinity. Further, when heated (800 to 1400°C), HA particles bind together to form sintered HA, and HA produced in this manner may be adopted for HA sprayed by plasma and flame on the implant surface. Even if HT-HA is processed into any form such as a dense body, porous body, particulate (granule) form, powder form, etc., it is impossible for it to undergo absorption or dissolution by the acid produced by osteoclasts. It is also called non-bioresorbable HA due to this property. On the other hand, LT-HA is produced by a mixture of various calcium phosphates, calcium carbonate and the like blended with a Ca/P ratio of 1.67, which gradually forms HA crystals under a low temperature environment the the solution. HA produced under such conditions is basically a nano-crystal, and is lower in crystallinity than HT-HA. Although LT-HA hardens by crystal formations, the porosity thereof becomes extremely large in comparison to that of sintered HT-HA. Having the above characteristics, LT-HA has the property of being absorbed by osteoclasts, so it is also called biodegradable HA. Incidentally, although the size of HA for bone or dentin is about 2.5 to 7.5 nm in width and about 10 to 30 nm in length, at this degree of crystal size, resorption by osteoclast is sufficiently possible even if there is some difference in size. This makes it applicable as a bone graft material because bone replacement by bone remodeling is possible. However, even LT-HA becomes non-resorbable when it becomes highly dense and crystalline like enamel due to crystal growth. From the above perspective, it is considered that the HA used for HA implants should be non-bioresorbable instead of biodegradable or bioborlabile, and HT-HA, that expresses osteoconductivity by bioactivity, is considered suitable for HA implant.

Taking into account the in vivo behavior of HA as described above, HT-HA that has osteoconductivity due to bioactive/ non-bioresorbable properties is considered suitable for HA coating, and low temperature or low crystalline HA is presumed to be suitable as a bone grafting material with bone replacement characteristics due to its bioactivity/bioresorbability. Therefore, whether the principle advantage of HA coating is osteoconductivity or bone substitution depends on the report, and it appears that unification of the theoretical basis has not yet been achieved.

Various techniques have been devised and put to practical use as methods for coating the Ti base metal surface with HA, but another issue with respect to HA implants is that the formation of a coating layer composed only of HA on the implant surface is difficult due to various technical problems. For example, when coating fired or sintered HT-HA to the surface of an implant in a high temperature environment such as by plasma or flame spraying, the production of oxyapatite by substitution from OH to O in the HA crystal near the surface layer or formation of calcium phosphates except HA by melting, destruction or denaturation etc. is likely. Therefore, even if an implant is labeled as being HA coated by thermal spraying, it seems difficult for the ratio of HA in the surface layer to exceed 70%, so it is assumed that recrystallization treatments are applied to the surface of the HA implant when necessary for the product. Vacuum heat treatment and pressurized hydrothermal treatment applied to this recrystallization and although the temperatures is less than that for high temperature HA produced by the firing operation, the HA produced by these treatments is not low-temperature type but is considered to be classified as HT-HA.

In addition, methods of depositing HA on coating from Ca-P solutions etc. and methods of depositing HA by sputtering etc. have been reported, but there are almost no scientific reports on whether or not products by
these reported methods have a sufficient component composition to be referred to as HA. In addition to the above, analytical data for the HA coating has only been for the entire coating layer up to now, and almost no information other than SEM observations can be found that shows information limited to the surface directly in contact with the vital tissues. Furthermore, analysis by XRD and other qualitative methods seem not to consider the surface or surface layer by analysis that extends to the inside of the layer. Furthermore, there are products in which Ti and Ti compounds are mixed in the HA coating layer itself or products without sufficient HA coating thickness in which the base metal is exposed. Various reports have been made that place these in the category of HA implants, and a valid evaluation of HA implants may not have been carried out as a result. That is, it is clear that it is difficult to express osteoconductivity/oстеointegration due to bioactivity unless the surface of the implant directly in contact with the bone is HA, and the characteristic as an HA implant is lost.

The AFM observation was conducted with the purpose of confirming the HA crystal on the coating surface, but when HA is highly sintered or when the crystal density is extremely high, it becomes difficult to observe the crystal itself. However, the direct confirmation of HA on the implant surface is clearly an important factor in the verification of an HA implant, so observation at the nano-level order was carried out. Comparing the structures of Implant-A and Implant-B, in which HA crystals were clearly observed by AFM on the surface of the coating, the whole surface of Implant-A had a vivid and distinct form at low magnifications, whereas B showed a relatively smooth and unclear form. This difference in form such as bulky, particulate, and block type sintered HA may have been caused by the difference in temperature and heating time when thermally spraying HT-HA on surface of the implant base metal. Since it is reported that Implant-A uses flame spraying, and B uses plasma spraying to bind HT-HA to the base metal surface, the shape deformation/denaturation may have occurred slightly more in B due to the higher spraying temperature.

Since the additional heat treatment is performed on the surface of Implant-A at a temperature of about 600 to 700°C under vacuum after flame spraying, HA crystals are reproduced on the surface by Ca-P originally denatured from HA. The reason for heat treatment in a vacuum environment is to prevent the production of TiO₂ (rutile) crystals produced by oxidation at high temperature on the base metal surface and which tend to detach or break down. Through undergoing such a process, the HA crystal observed on the surface of A seemed to exhibit a regular arrangement/orientation that has a certain directionality, which is one of characteristics of HT-HA. Also, in addition to an intermingling of sections showing a distinct crystalline form and a somewhat ambiguous form, the formation region of the observed HA itself was partial and the other sections were amorphous or molten, and there were regions where no crystalline structure was observed. The reason for this was likely to be because melted and denatured Ca-P formed by flame spraying was observed, and it was partially precipitated as HA from Ca-P by the heat treatment. Therefore, it is assumed that many regions on the surface of A are covered with Ca-P or Ca compound rather than HA. Similar results were also shown in the analysis of the surface by XPS. However, the rough surface may be related to the reason that the XPS waveform for each HA element of A showed a tendency to be smaller than in the other samples. In addition, in the analysis of the surface layer by micro-XRD, a strong diffraction pattern of Ti and small diffraction pattern of Ti oxide were detected and these may be due to the base metal itself and oxidation of the metal by the high temperature treatment. Also, the diffraction pattern of HA was relatively low, and the crystallinity also differed from the typical pattern of the control HA. Although the HA seen on the surface of A showed good crystallinity, amorphous Ca-P etc. were probably present due to decomposition, melting, denaturation etc. of HA by spraying or heat treatment, etc. Therefore, the HA surface coverage was partial and the HA content in the surface layer was also partial. However, since the clear presence of HA on the surface was confirmed, it was basically inferred that Implant-A might exhibit good biocompatibility and osteoconductivity.

In contrast, HA crystals were observed on the whole surface area of Implant-B, and no regularity was observed in the alignment and orientation of the crystals. HA crystals were precipitated and produced on the surface by treating the HA plasma spray surface with a Ca-P amorphous calcium phosphate (ACP) solution at 450 to 500°C under pressurized conditions. The crystals were classified as high temperature type according to the production temperature, crystal shape and crystallinity, and it was assumed that the crystals showed irregular arrangement/orientation due to precipitation from solution. Since substances other than HA and a small quantity of Ca-P were not found on the surface and surface layer of B, the coating was nearly completely covered with HA. In particular, since analyses of the top surface by XPS and micro-XRD gave waveforms and diffraction patterns almost identical to the HA control, the top surface of Implant-B showed high crystallinity of HA and the coated state was excellent. In XPS and micro-XRD analyses, CaCO₃ was detected in a small quantity, but it was inferred that the Ca/P ratio of B was larger than that of the HA control due to the presence of CaCO₃. In other words, Implant-B might exhibit excellent biocompatibility and osteoconductivity due to the confirmation of the HA crystal in almost the entire area of the surface and the clear detection of HA from the surface/surface layer.

However, in addition to the shape and size, there are differences in the arrangement/orientation of HA crystals present in vivo conditions, due to the difference in the blastodermic layer of the hard tissue. The enamel HA derived from the ectoderm and constantly exposed to the intraoral environment has a crystal size of 100 to 1000 nm in length and 30 to 60 nm in width, and has high crystallinity. In contrast, dentin, cementum and bone HA crystals derived from the mesoderm are small at 30 to 50 nm in length and 10 to 30 nm in width, and have low crystallinity. Enamel with high crystallinity and density of HA shows a regular arrangement/orientation structure in which c-axis as the principal axis of HA is oriented vertically to the enamel surface and the c-plane is facing the surface, and this is closely related to HA showing excellent physical strength in the c-axis direction. Also, the a-b plane in which HA is formed by the columnar axis Ca²⁺ of the crystal potential is slightly positively charged, so negative charged substances are easily adsorbed. In contrast, since PO₄³⁻ and OH⁻ are present on the c-plane facing the axis, positive charged substances are easily adsorbed due to it being negatively charged. That is, since the c-plane of HA is oriented vertically to the enamel surface, the surface potential is negatively charged and an increase resistance to acid and growth due to Ca²⁺ bond in the c-axis direction occur easily. In addition, it has a structure that constantly mitigates the adsorption of bacteria that was continually negatively charged. Enamel derived from the ectoderm is in an environment where it is always exposed to the infection and contamination in the oral cavity. However, it is oriented with the c-plane facing the surface, and it seems to respond to the resistance to decalcification due to dental decay etc. by increased crystallinity and density. In contrast, the dentin, cementum and bone of hard tissues derived from the mesoderm should be present in the interior of the body, and HA crystals in these tissues are infinitesimal and have low crystallinity unlike enamel. Although their arrangement also tends to be
oriented in the weighted direction caused by cellular mechanosensing, they basically do not have strong regularity. Therefore, since no specific electric charge is generated in any part of each tissue in the in vivo environment, the surface exhibits clearly different properties from the enamel. Taking into account the above and assuming HA is in contact with bone tissues, HA with no regularity in arrangement/orientation may be preferable in the case where osteoconductivity occurs due to the bioactive property of HA. In light of this, it is of great clinical interest that the HA seen in Implant-A showed a regular arrangement/orientation close to that of enamel, while the HA of Implant-B showed an irregular arrangement/orientation close to that of bone.

AFM observations of the surfaces of Implant-C, Implant-D and Implant-E showed no clear HA crystals, but the presence of HA was clearly confirmed by XPS and micro-XRD. First, concerning Implant-C, it has been reported that a thin film coating layer of HA is formed by a process called thermal decomposition treatment after applying a Ca-P solution to the base metal surface and drying it, but it is not clear from the production method whether this is classified as HT-HA or LT-HA, and as described above, the HA crystal structure was not observed by the AFM. Although Implant-C was reported as having a calcium titanate (CaTiO$_3$) layer as the undercoating of the HA layer, it was difficult to clearly distinguish between CaTiO$_3$ and HA layers consisted of 3 to 5 μm thickness, and was assumed to be partly mixed. Therefore, the reason why the detected intensities and the waveforms of the element for Implant-C by XPS were similar to those of the control may involve the presence of CaTiO$_3$ and Ca-P, in addition to the presence of HA on the surface. Although the presence of HA was confirmed, even though the diffraction pattern by micro-XRD for the surface layer part was weak, the crystallinity was low given the diffraction pattern. That was, confirmation of the clear intensity and waveform of amorphous TiO$_2$ by XPS for the surface and the detection of strong intensities and distinct diffraction patterns for Ti and Ti oxides by micro-XRD suggested that the coating itself was not mainly consisted of HA. Furthermore, since the intensities and waveforms of the HA-related elements detected by XPS were also likely to be detected from elements contained in substances other than HA, the ratio of the presence of HA in the surface of Implant-C might contain Ti and Ti compounds in parts. Based on the above results, Implant-C had the biotolerant and bioinert properties of Ti and Ti compounds in addition to bioactivity due to HA, so although Implant-C might show good biocompatibility and osteoconductivity due to the bioactive property of HA, osteoconductivity attributed to the bioactivity of HA might decrease somewhat.

Regarding Implant-D, although some Ca-P-like sites were observed on the surface by AFM, clear HA crystal could not be confirmed. But the existence of HA was confirmed by XPS and micro-XRD analyses, and Ti and a small quantity of TiO$_2$ was also confirmed by the same analyses. Since D is considered to have a thin HA coating layer, the possibility of detection of Ti/TiO$_2$ from the base metal of the implant was assumed. The HA coverage on the surface was partial and it seemed likely that Ca-P other than HA was also present. It was reported that the HA coating area and the exposed titanium portion existed together on the surface of D, and osteoconductivity of HA and cell adhesiveness of Ti could be obtained by having such a double surface. However, clear results on the substantial effect have not been sufficiently indicated. Actually, Ti exposed surface was observed by AFM, but the cell adhesiveness of Ti was the different property from the bioactivity of HA which signified osteoconductivity, as well as the inherent biotolerance of Ti, making it difficult to judge to what category it belonged. According to the manufacturer, Implant-D had a thin HA film of about 1 to 2 μm thickness that was unlikely to detach, bone resorption hardly occurred in comparison to other implants, and it had excellent long-term stability. However, judging from the results of this study, although Implant-D might exhibit relatively good biocompatibility and osteoconductivity due to the bioactive property of HA and biotolerant property of Ti, the osteoconductivity attributable to the bioactivity of HA might decrease somewhat, similar to Implant-C.

It has been reported that Implant-E has an HA coating layer formed of various shapes by treating the base metal surface with a Ca-P solution at low temperature. As mentioned in the results, clear HA crystals were not observed on the surface, but in addition to confirming the clear presence of HA by XPS and micro-XRD analyses, the detection of almost no substances other than Ca-P indicated that the crystallinity and content of HA was excellent. However, judging from the manufacturing process of Implant-E, it seemed that the HA of E belonged to LT-HA differed from HT-HA. Still, even if it was LT-HA, the results of XPS and micro-XRD indicated that the crystallinity and the coated state were excellent. These results indicated that Implant-D might show good osteoconductivity due to excellent bioactivity of HA. As described above, the samples in which clear crystals observed on the surface were only Implant-A and Implant-B, and crystals were not observed in the others due to decomposition and denaturation of HA caused by heating, or other Ca-P formation. In addition, the top surface of Implant-B and Implant-E were determined to be sufficiently composed of HA, and judging from the constituent components, mainly comprising components other than HA were confirmed in the others. However, since there were the consideration of relative comparison with the HT-HA used as a control, these results and discussion were not definitive and further data and analysis might be necessary. Still, there were many unclear aspects concerning the HA coating layer, and this incomplete but partial clarification of the actual state of the HA coating layer could be an indicator for clinical applications in the future. As mentioned above, HA has been applied to implant coatings because of its superior osteoconductivity attributable to its excellent bioactivity. However, clinical application under the name of HA coating without indicating clear criteria for the HA implant may lead to the erroneous assessment of HA. Taking this into account, there seems to be an urgent need to investigate and organize the necessary criteria for HA coatings.

In this study, AFM observations, and XPS and micro-XRD analyses were performed on the top surface of five commercially available HA implants, leading to the following conclusions: HA was demonstrated to be present on the surface and in the surface layer of the HA coating in all the samples. AFM showed that HA crystals were clearly observed on the surface of Implant-A and Implant-B. XPS and micro-XRD analyses exhibited that HA was detected in the HA coating layer of all the samples, but other substances including Ti, Ti oxides, etc. were also confirmed in Implant-A, Implant-C, and Implant-D. In general, all samples were suggested to exhibit good biocompatibility and osteoconductivity due to their bioactive properties caused by HA.

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Conflict of Interest
The author have declared that no COI exists.

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