Analysis of Crystal Structure of Bone Graft Material Using Analyses of X-Ray Diffraction and Scanning Electron Microscope Image

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Objective: The present study was to analyze the crystal structure of Bio-Oss, the representative bone graft material, through X-ray diffraction (XRD) and scanning electron microscope (SEM) image analyses.

Methods: Xenogeneic bone (Bio-Oss; Geistlich Biomaterials, Wolhusen, Switzerland) for bone graft of which particle size was in the range of 0.25-1.0 mm was used for the present study. Specimens were quickly frozen and then pulverized to conduct XRD analysis. A SEM was used to observe materials in forms of powder and monolith, respectively.

Results: The XRD pattern of the specimen showed components of bone and hydroxyapatite (HA). The broad peak appearing in the vicinity of 12.6° was seen as organic material. In the SEM, pores were observed together with the coagulation of small particles. Particles were concluded as those of small HA with sizes of around 50 nm.

Conclusion: Xenogeneic Bio-Oss consist of apatite of low crystallinity similar to the bony tissue of humans. Thus, consequences of bone graft employing Bio-Oss are expected to be different from those of bone graft using bone graft materials of high crystallinity regarding the healing process.

Keywords: apatite, bone, regeneration, hydroxyapatites, implants

Introduction

An ideal material for the implant of artificial teeth would be an autologous bone. Autologous bone graft would be the most ideal one for implanting artificial teeth because osteoblasts known to be in charge of osteogenesis and induce bone regeneration are contained inside the autologous bone where the structure can act as an excellent bone conductive material. However, autologous bone imposes a burden of collecting part of one’s own body. It is disadvantageous due to its easy absorption in the stage of healing after bone graft [1,2]. Allogenic or xenogeneic bones including substitute bones have been developed and introduced into clinics to overcome such disadvantages. However, materials having the capacity of osteogenesis equal to autologous bone have not been developed yet.

Materials that are capable of substituting autologous bone, such as bovine bone mineral, Bio-Oss (anorganic bovine graft), demineralized bone matrix, Drilac (polylactic acid gettyimages"

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granules), hydroxyapatite (HA), poly(lactide-co-glycolide), microparticles synthetic biodegradable polymer, and tricalcium phosphate (TCP) have been reported [3-5]. Recently, Bio-Oss (Geistlich Biomaterials, Wolhusen, Switzerland), the xenogeneic bone which is most similar to autologous bone, and the β-TCP, the synthetic bone, are used in clinics frequently. Bio-Oss is a HA extracted from the matrix of bovine bone. It is used as the most representative xenogeneic bone graft material for which the organic substance therein is removed through specific treatment process. It is porous similar to human bone, thus enabling easy vascular generation. It is also structurally stable and resistant against absorbance. Therefore, it has been employed in clinics since 1980s as a material for bone graft [6-9].

It has exhibited comparatively favorable consequences in clinics. Thus, it has been employed frequently. However, information on the material of bone graft is insufficient. Thus, the objective of the present study was to analyze crystal structure of Bio-Oss, the representative bone graft material, through X-ray diffraction (XRD) and scanning electron microscope (SEM) image analyses.

Materials and Methods

The commercially available xenogeneic bone (Bio-Oss) for bone graft of which particle size was in the range of 0.25-1.0 mm was used for the present study. Specimens were quickly frozen and then pulverized to conduct XRD analysis. These pulverized specimens were placed on the holder of analysis using an XRD analyzer (D8 Advance; Bruker AXS GmbH, Karlsruhe, Germany) at 2θ range of 10° to 80°.

To examine the microstructure on the surface of specimen, a SEM (S-4200; Hitachi, Tokyo, Japan) was used to observe materials in forms of powder and monolith, respectively.
Results

The XRD pattern of specimen showed components of bone and HA. The broad peak appearing in the vicinity of 12.6° was seen as an organic material, the amorphous calcium phosphate present in the bone (Figure 1).

The SEM image of the specimen of monolith rendered bony tissue at lower level of magnification, whereas pores of the level of submicron were observed together with coagulations of small particles in the form of suspension of pulverized powders.

Particles were concluded as those of small HA with sizes of around 50 nm. They were commonly observed from the two specimens (Figure 2).

Discussion

Upon damages to bone by trauma or by operation, or in the case of artificial bone graft onto the part of such damages, the generation or regeneration of bone due to interactions between osteoblasts and osteoclasts would occur.

Such healing process of bone is comprised of the following: 1) the stage of inflammation, wherein the reaction of inflammation arises in the part of bone graft or bone damages within the first two weeks and results in re-vascularization; 2) the stage of Phase 1 Bone Regeneration of following period of the 3rd and 4th week, wherein the callus and woven bone are generated through the process consists of osteoinduction, osteoconduction, and osteogenesis, and 3) the stage of Phase 2 Bone Regeneration, comprising the resorption of callus and woven bone which were created in the stage of Phase 1 Bone Regeneration, wherein the remodeling of bone occurs by replacement of callus and woven bone through resorption during the following period extending from 6 weeks to few months [10].

The new bone generated by bone graft through the process of Phase 1 Bone Regeneration becomes mineralized and hardened bone in the formation of lamella configuring matured haversian system that endures external pressure or functional forces.

This is why bone graft onto parts of damages in bone is executed to secure safe and stable prognosis of the implant of artificial teeth in dentists’ clinics. Analysis of XRD is used for the analysis of crystal structure of solids by examining patterns of XRD of pulverized specimens.

Crystal structures of specimens of identical chemical formula could differ from each other. XRD analysis can be employed to determine the size and structure of crystals. For the estimation of size of crystals, the Sherrer’s equation is used. Values of 2-threta with regard to the peak appearing from each form of HA and β-TCP etc. are constant. The database called Joint Committee on Powder Diffraction Standards holds locations and comparative sizes of each mineral. By referring to the measurement of XRD of bone graft and locations in the database, we can determine the structure and mass of subject material. Wider and narrower XRD peaks signify low and high crystallinity of the subject material, respectively [11,12]. In the present study, the peak of Bio-Oss showed the aspect spread over sideward comparing to the known XRD pattern of HA. This signifies the low crystallinity and smaller sizes of the domain of crystal [1].

The apatite present in bony tissue forms the shape of ceramic/polymer nanocomposite [13]. The apatite present in bony tissue of humans exhibits low crystallinity with particle sizes distributing in the range of dozens of nanometers. On the contrary, the HA manufactured through the process of sintering at high temperature exhibits high crystallinity wherein the grain growth would result in dozens of the size of crystals compared to apatite existing in bony tissue. In the case of the degree of high crystallinity and bigger size of particles, in vivo biodegradation by osteoclasts would be almost impossible with very low capability of bone conduction. The low crystalline carbonic apatite exhibits the most excellent effect of bone conduction [14]. In the present study, it was identified that the Bio-Oss was composed of low crystalline apatite. This suggests the relevance to comparatively favorable clinical consequences of operations of bone graft with the material.

However, other studies have reported disadvantages of Bio-Oss compared to other synthetic bone due to insolubility of Bio-Oss. There are no direct evidences showing inhibition of symphysis of implants by remaining bone graft materials despite significant amount of grafted materials unabsorbed from the operation of the implant of artificial teeth upon completion of bone graft. Rather, the survival rate of implant could increase due to significant increase in bone density [15-19].

In this study, we also performed energy dispersive spectrometry (EDS) and Fourier transform infrared spectrometer (FT-IR) analyses, however, no significant results were obtained. Because the sample volume was small, and the progress was not desired.

In the EDS study, as C was continuously observed at about 10wt%, it could be confirmed that there was a possibility that organic matter was present in the sample or carbonate ion was coordinated with calcium instead of phosphoric acid.

In the FT-IR analysis, a bending vibration equivalent to CO$_3^{2-}$ could be observed, confirming that only some carbonate ions could be present in the sample.

More careful experiments and comparative analysis with...
other graft materials will be needed in the future.

The formation of newly generated bone after grafting of substitutional substances for bone follows processes of osteogenesis, osteoinduction, and osteoconduction. Osteogenesis is a process activated by osteoblast and bone matrix inside the bone graft material, whereas bone induction refers to the process of inducing differentiation of osteoblast by undifferentiated mesenchymal cell after chemotactic reaction by bone graft material on host. Osteoconduction is a process of deriving osteoblasts into adjacent bone graft material from the surface of the bone of host wherein the bone graft material plays the role of a scaffold [20].

Ideal materials to be substituted for bone should be bio-compatible and can prevent immunoreaction of host. Bone graft materials should be absorbed and replaced with newly generated bone as time goes by. The grafted material should have properties of osteoinduction and osteoconduction for the formation of new bone [21]. Principles behind the formation of new bone have been explained in the literature. However, the action mechanism of bone regeneration at cell level and the most feasible material for bone substitution to be employed in clinics are yet to be clarified.

Several factors can cause bone resorption around the implant [22]. To overcome this problem, the bone graft is performed. In alveolar bone defects that are often seen in post-menopausal women, bone regeneration through membranes and bone grafts is essential for an implant or periodontal treatment [23,24]. Ideally, this bone regeneration will help maintain healthy periodontal tissue that can minimize future disease outbreaks.

Therefore, the search for ideal material for bone graft seems necessary through further studies, although Bio-Oss has demonstrated comparatively favorable clinical consequences.

**Conclusion**

Bio-Oss consist of apatite of low crystallinity simillar to the bony tissue of humans.

Thus consequences of bone graft employing Bio-Oss are expected to be different from those of bone graft using bone graft materials of high crystallinity regarding the healing process.

**Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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**References**