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Analysis of the Inorganic Component of Autogenous Tooth Bone Graft Material

Young-Kyun Kim[1], Su-Gwan Kim[2,*], Ji-Su Oh[2], Seung-Chan Jin[2], Jun-Sik Son[2], Suk-Young Kim[3], and Soo-Young Lim[3]

[1]*Department of Oral and Maxillofacial Surgery, Section of Dentistry, Seoul National University Bundang Hospital, School of Dentistry, Seoul National University, Seongnam 463-707, Republic of Korea*
[2]*Department of Oral and Maxillofacial Surgery, School of Dentistry, Chosun University, Gwangju 501-759, Republic of Korea*
[3]*School of Materials Science and Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea*

This study was performed to identify the calcium phosphate minerals, chemical element and Ca/P ratio and to examine the surface structure of autogenous tooth bone grafting material (AutoBT) which recently developed and applied clinically as a bone graft materials. The analytical results showed that AutoBT is composed of low-crystalline hydroxyapatite (HA) and possibly other calcium phosphate minerals, which is similar to the minerals of human bone tissues. And the dental crown portion was composed of high-crystalline calcium phosphate minerals (mainly HA) with higher Ca/P ratio while the root portion was mainly composed of low-crystalline calcium phosphates with relatively low Ca/P ratio.

Keywords: Bone Graft Material, Calcium Phosphate, Tooth.

RESEARCH ARTICLE

1. INTRODUCTION

Since 1993, we have conducted experiments to develop bone graft materials using human teeth, and we have obtained a Korean patent. Later, the development of bone graft materials using animal teeth was obtained a U.S. patent. The bone graft materials developed using the animal or human teeth were termed tooth-ash. The tooth-ash mainly consisted of a hydroxyapatite (HA) mineral.[1–7]

Based on the results of the previous studies, autogenous tooth bone graft materials (AutoBT) were commercialized in 2008, and the positive clinical results have been reported.[8] Therefore, this study was conducted to provide more detail information on AutoBT materials to clinicians in terms of their mineral composition as well as their surface structure.

2. EXPERIMENTAL DETAILS

2.1. Materials

Teeth extracted from patients that had been stored in ethyl alcohol and consigned to the Korea tooth bank. In order to analyze the surface structure and mineral components of the teeth, each tooth was divided into the two parts: the crown and the root. The crown consisted of enamel and dentin and the root consisted of dentin and cementum.

The powder samples of the two parts were prepared as bone graft materials: fresh sample, and AutoBT powder. Fresh samples (sample 1) were prepared by removing the soft tissues attached to the extracted teeth. The soft tissue removed samples were freeze-dried. The AutoBT powder (sample 2) was prepared by removing soft tissues, washing, defattening, partial decalcification, and freeze-drying. The two samples were divided into the crown and root portions and kept in a drying oven at 100 °C.

2.2. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS) Analysis

The surface structure of enamel, dentin, and cementum of a human tooth was examined using SEM (S-4800, Hitachi, Japan). For SEM study, the surface of the fresh sample was coated with 7 nm thick platinum (Pt) coating.

The chemical element and Ca/P ratio of each part of the tooth were quantitatively examined using EDS (X'Pert PRO, PANalytica, Netherlands) attached to SEM. The samples (fresh sample and AutoBT) used for the analysis of the composition ratio were prepared without Pt coating.

*Author to whom correspondence should be addressed.

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2.3. X-ray Diffraction (XRD) Analysis

To examine the mineral crystalline phase of the two types of samples obtained from extracted teeth, the samples which were obtain the strong and detail XRD patterns. The samples were finely ground for XRD samples.

The both samples were inserted into a glass sample holder for the mineral phase analysis and measured by an XRD analyzer applying Cu ($\lambda = 1.5218$ Å) to 10–90° at the speed of 1 sec/step.

3. RESULTS AND DISCUSSION

3.1. Surface Structure

The SEM micrographs of the enamel, dentin, and cementum areas obtained from the fresh sample 1 are shown in Figure 1 with various magnifications. As shown in Figures 1(a1∼a3), the enamel surface of the crown portion was very smooth and dense in comparison with other portions. In addition, the surface holes were not detected in the enamel. In contrast with the enamel, many long columnar holes with an uniaxial direction were detected and uniformly distributed on the surface of the dentin portion (Figs. 1(b1∼b3)) and (Fig. 2). The surface of the root portion was covered by the cementum. The SEM micrograph of the root portion is shown in Figures 1(c1∼c3). Although long holes were not detected in the cementum, column-like materials were arranged vertically on the surface. This pattern on the cementum was formed by the organic-inorganic complex of low-crystalline minerals and collagen matrix. It is believed that the complex cementum structure plays an important role in the connection of dentin to adjacent soft tissues.

The surface revealed sharp and long HA crystalline feature. Indeed, enamel almost consists of HA mineral, which is a ceramic material with a high degree of hardness. In SEM (Fig. 3), the alignment of HA crystals appeared to have a certain direction, which may be related to the loading direction of teeth. The porous enamel layer at under the enamel top surface is thought to be HA mineral with holes which are related to dentinal holes in the dentin region of sample 2.

In SEM micrograph of the dentin of sample 2 (Fig. 4), many dentinal tubules and a dense collagen matrix were

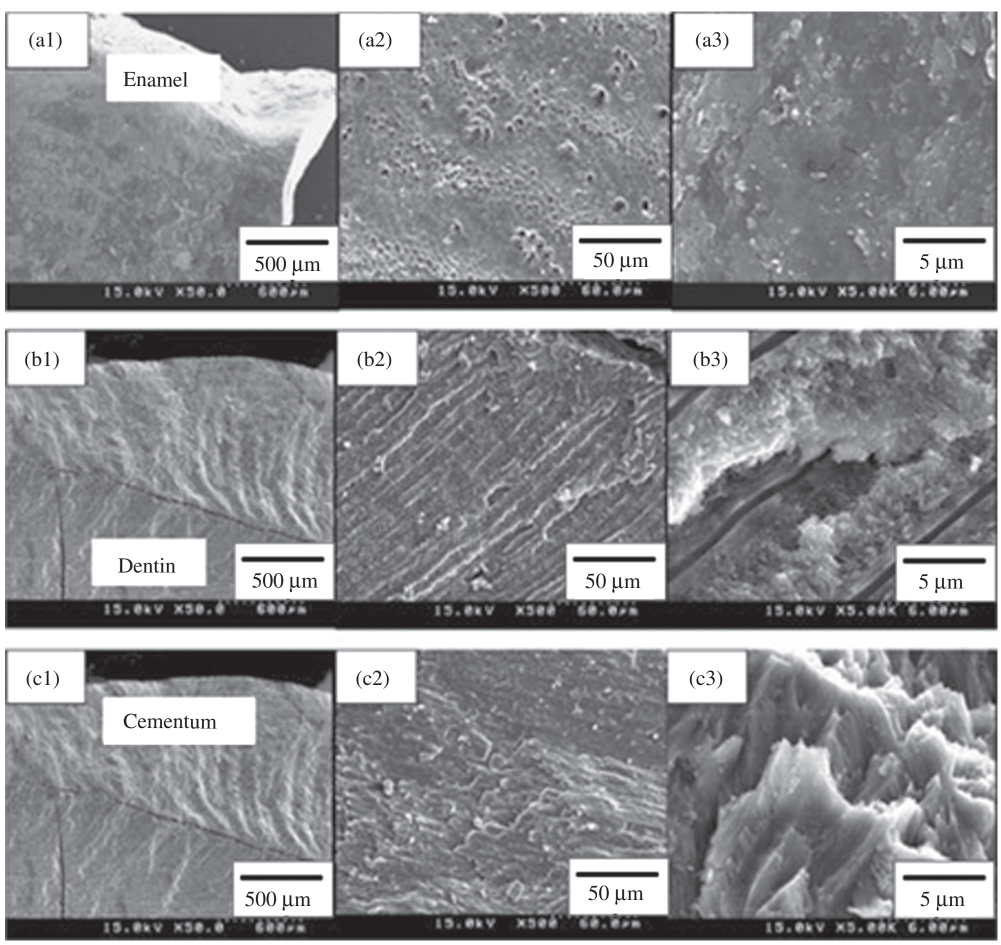

Fig. 1. SEM photographs of sample 1 of enamel (a1–a3), dentin (b1–b3) and cementum (c1–c3) regions of a human tooth with various magnifications (50, 500, 5000X).

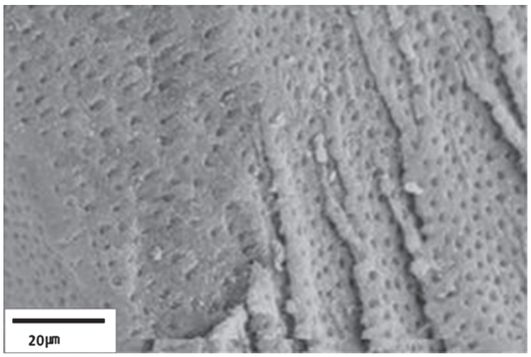

Fig. 2. SEM micrograph of the surface of dentin region of sample 1.

observed. The diameters of the dentinal holes were approximately 1∼2 μm, and they were arranged irregularly at approximately 5 μm intervals.

3.2. Surface Composition

The EDS results of the enamel part of fresh sample 1 showed that carbon accounted for approximately 56% of its composition, and the Ca/P ratio was found to be approximately 1.54. However, slightly more carbon was detected in both the dentin and cementum, compared with the enamel. And the Ca/P ratios were approximately 1.02 and 0.96, respectively (Table I).

Table II showed the result of the EDS analysis of the crown, root, and total tooth powder (sample 2). The Ca/P ratio of the total tooth were the range of 1.24∼1.46, which were comparable to the values of tricalcium phosphate (TCP) and octacalcium phosphate (OCP). The Ca/P ratio of crown portion was 1.75, which was similar to the HA value. The root portion had 1.32, which was similar to the value of amorphous calcium phosphate (ACP).

Each portion of human teeth showed the different Ca/P ratio (i.e., the crown primarily consist of enamel and is Ca/P=1.75, and the root consists of dentin and cementum and 1.32). Thus, the healing rate and mechanisms with the crown and root portions would likely be different when used for autogenous tooth bone graft materials.

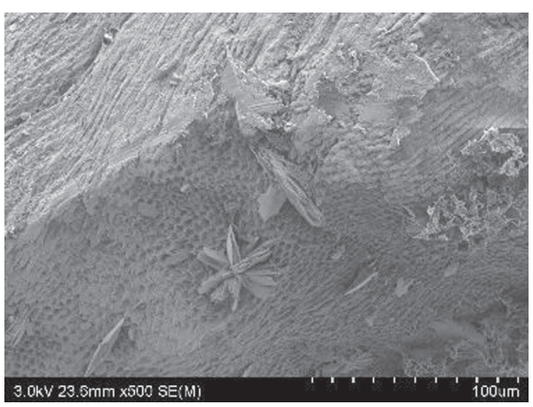

Fig. 3. SEM micrograph of the enamel surface of sample 2 (AutoBT).

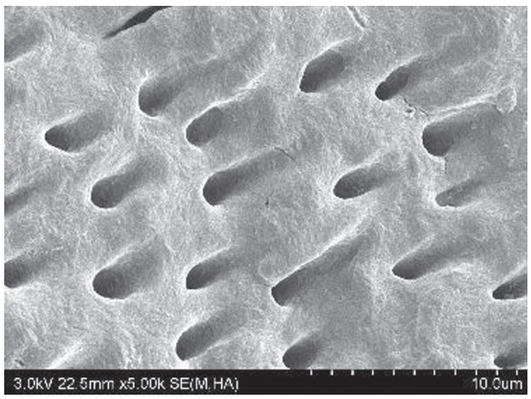

Fig. 4. SEM micrograph of the dentin surface after partial demineralization (sample 2).

3.3. XRD Analysis

The crown of fresh tooth sample 1 was composed of almost 100% HA with relatively high crystallinity. The results of the XRD analysis showed TCP in addition to HA. The results of the quantitative XRD analysis showed that HA (ICSD-022059) accounted for 88.2% and TCP (ICSD-6191) accounted for 11.8%.

Similar to the crown, the XRD analysis of root showed almost 100% HA crystalline. But the XRD peak in the root was wider than the crown, which suggested that the crystallinity of a root is low. Nevertheless, the root showed high crystalline HA (98.8%) and 1.2% ß-TCP, which were similar to the crown. Because the total tooth consisted mainly of dentin, it was composed of low-crystalline HA, which was similar to the root.

Table I. EDS results of each region of the human tooth (sample 1).

	Enamel	Dentin	Cementum
Element	At%	At%	At%
C	56.17	64.19	65.72
O	40.86	30.53	29.89
P	1.17	2.61	2.24
Ca	1.80	2.67	2.16
Ca/P	1.54	1.02	0.96

Table II. EDS results of each region of the human tooth (sample 2).

	Total tooth	Crown	Root
Element	At%	At%	At%
C	60.65∼65.72	12.21	64.19
O	29.89∼31.48	55.66	30.53
P	2.24∼3.69	13.65	2.61
Ca	2.16∼4.18	18.49	2.67
Ca/P	1.24∼1.46	1.75	1.32

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In order to examine the mineral components of the AutoBT powder (Sample 2), XRD was performed separately on the upper crown portion and the lower root portion. In both portions of the tooth, the primary pattern was HA, but the presence of small amounts of ß-TCP, ACP, and OCP were also confirmed. However, the level of HA crystallization and the amount of HA differed greatly depending on the area of the tooth. The XRD pattern was much stronger in the crown portion with enamel than in the root portion. These results were in agreement with the results recently reported by Xue et al.[9]

In general, tooth mineral components consist of five biologic calcium phosphates (HA, TCP, OCP, ACP, and DCPD). Researchers have speculated that the five calcium phosphates react mutually, which means that favorable remodeling could occur *in vivo* grafts.[10] In our analysis of the inorganic components of AutoBT, the presence of HA (Ca/P = 1.75), TCP (Ca/P = 1.46), ACP (Ca/P = 1.32), and OCP (Ca/P = 1.24) in AutoBT means that favorable remodeling could occur *in vivo* grafts. Thus, it is expected that the bony healing and remodeling by AutoBT grafting can be similar to the result achieved by autogenous bone grafting.

We have designed additional studies to improve the physical and chemical properties of AutoBT as well as HA stability through the application of nano-technology in the future.[11, 12]

4. CONCLUSION

AutoBT are composed of low-crystalline HA and possibly other calcium phosphate minerals, which is similar to the minerals of human bone tissues. The similar mineral components of AutoBT may be showed the compatible biological property with autogeneous human bone. When AutoBT is grafted, it is expected that the rate of bony healing will be excellent and the pattern will be varied depending on grafted tooth portion, such as crown or root.

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