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Development of a novel bone grafting material using autogenous teeth

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We developed a novel bone grafting material that incorporates autogenous teeth (AutoBT), and provided the basis for its clinical application. AutoBT contains organic and inorganic mineral components and is prepared from autogenous grafting material, thus eliminating the risk of an immune reaction that may lead to rejection. AutoBT was used at the time of implant placement, simultaneously with osteoinduction surgery, and excellent bony healing by osteoinduction and osteoconduction was confirmed. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:496-503)**

Diverse biomaterials have been used in dental surgery and, with continuous research and development as well as academic advancements, a variety of new biomaterials have been commercialized. In oromaxillofacial surgery, periodontal surgery, implant surgery, and diverse other fields, grafting biomaterials are used to repair hard and soft tissue defects, in conjunction with guided tissue regeneration and guided bone regeneration, and in esthetic and reconstructive plastic surgery.

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Autogenous bone is an ideal material for the reconstruction of hard tissue defects, because it promotes osteogenesis, osteoinduction, osteoconduction, and rapid healing, but it does induce immune rejection. However, the disadvantages of autogenous bone as a grafting material are that the harvest volume is limited, resorption is unavoidable, and a second defect is induced in the donor area. To overcome these limitations, allogeneic bone, xenogeneic bone, and synthetic bone have been used in clinical practice; nevertheless, efforts have continued to develop more ideal bone grafting materials.[1] However, owing to concerns regarding the spread of infection and the high cost associated with allogeneic or xenogeneic bone, clinicians and patients may opt against these sources of grafting material. Synthetic bone, in contrast, is relatively inexpensive and involves no risk of disease, but it lacks the ability to promote osteogenesis and osteoinduction, and thus its utility is limited for the formation of viable bone.

We have been conducting research on the development of biomaterials using human teeth since 1993, and we recently reported the results of several of our advanced studies.[2-23] We obtained a Korean patent based on this research, and obtained an American patent for developing bone grafting materials using animal teeth.[24,25] Furthermore, the feasibility of repairing hard tissue defects using bone grafting material

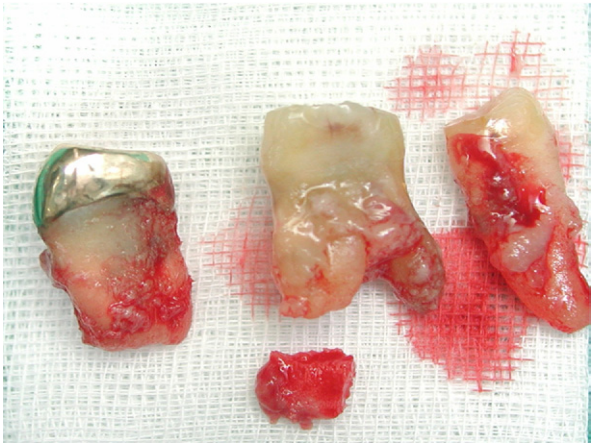

Fig. 1. Extracted teeth.

Fig. 2. Contaminants attached to the extracted teeth were removed, and bone grafting material was prepared as a syringe type using a nondecalcifying method.

derived from animals, in which the major components are hydroxyapatite (HA) and human tooth ash, has been demonstrated experimentally.[26,27]

Based on previous studies, we focused on the development of a new bone grafting material that promotes bone regeneration but overcomes the limitations of autogenous, xenogeneic, and synthetic bone. Here, we show that the use of AutoBT, a novel bone grafting material produced from autogenous teeth, resulted in excellent bone healing based on an analysis of its inorganic components, surface structure, and histologic evidence of the healing process in harvested specimens after clinical application.

MATERIALS AND METHODS

Treatment and grafting method using AutoBT

Extraction. When the decision was made to extract teeth that could not be preserved, the treatment process using autogenous teeth was explained to the patient. If the patient was willing, he or she provided informed consent to the treatment and use of the extracted teeth.

Storage and preparation of the extracted teeth. The extracted teeth were placed in a storage container and stored in a refrigerator or freezer. Dentists prepared a document assigning the teeth for use in AutoBT treatment according to the desired particle size (0.5-1 or 1-2 mm in diameter), depending on the intended use.

AutoBT treatment (Figs. 1 and 2). The teeth were crushed to a powder after first trimming off the soft tissues. The size of the particles was between 200 and 1,000 μm in diameter, although it is possible to use particles >1,000 μm in diameter. Contaminants and the remaining soft tissues were removed by washing the crushed tooth powder. Next, the washed autogenous tooth was subjected to a dehydration and defatting process and then lyophilized. After sterilization with ethylene oxide, the powder was packed and transported to the hospital where implant surgery would be performed.

Transplantation and storage. The resulting powder was used by dentists to perform bone grafts during implant surgery. For those who will likely require future surgeries, the powder can be stored at room temperature for up to 5 years. Because specialized facilities are not required, the powder can be stored at the patient's medical institution or in the patient's home.

Analysis of inorganic components

The prepared AutoBT sample was inserted into an analytic glass holder, and the diffraction pattern was measured using an X-ray diffraction analyzer (X'Pert Pro MPD; Panalytica, Almelo, The Netherlands) with Cu radiation ($\lambda = 1.5218$ Å) over 10°-60° at a rate of 1 s/step.

Scanning electron microscopy

A scanning electron microscope (S-4800; Hitachi, Ibaraki, Japan) was used to examine the surface structure of AutoBT powder. For scanning electron microscopy (SEM), the surface of the sample was coated with a 7-nm-thick platinum (Pt) coating, and the sample was examined at ×400, ×5,000, and ×10,000 magnification.

Clinical cases and histomorphometric analysis

We treated a 40-year-old male patient with an abscess near the root apex of the left maxillary first premolar tooth (Fig. 3). The affected tooth was extracted, processed into AutoBT powder, and, after 2

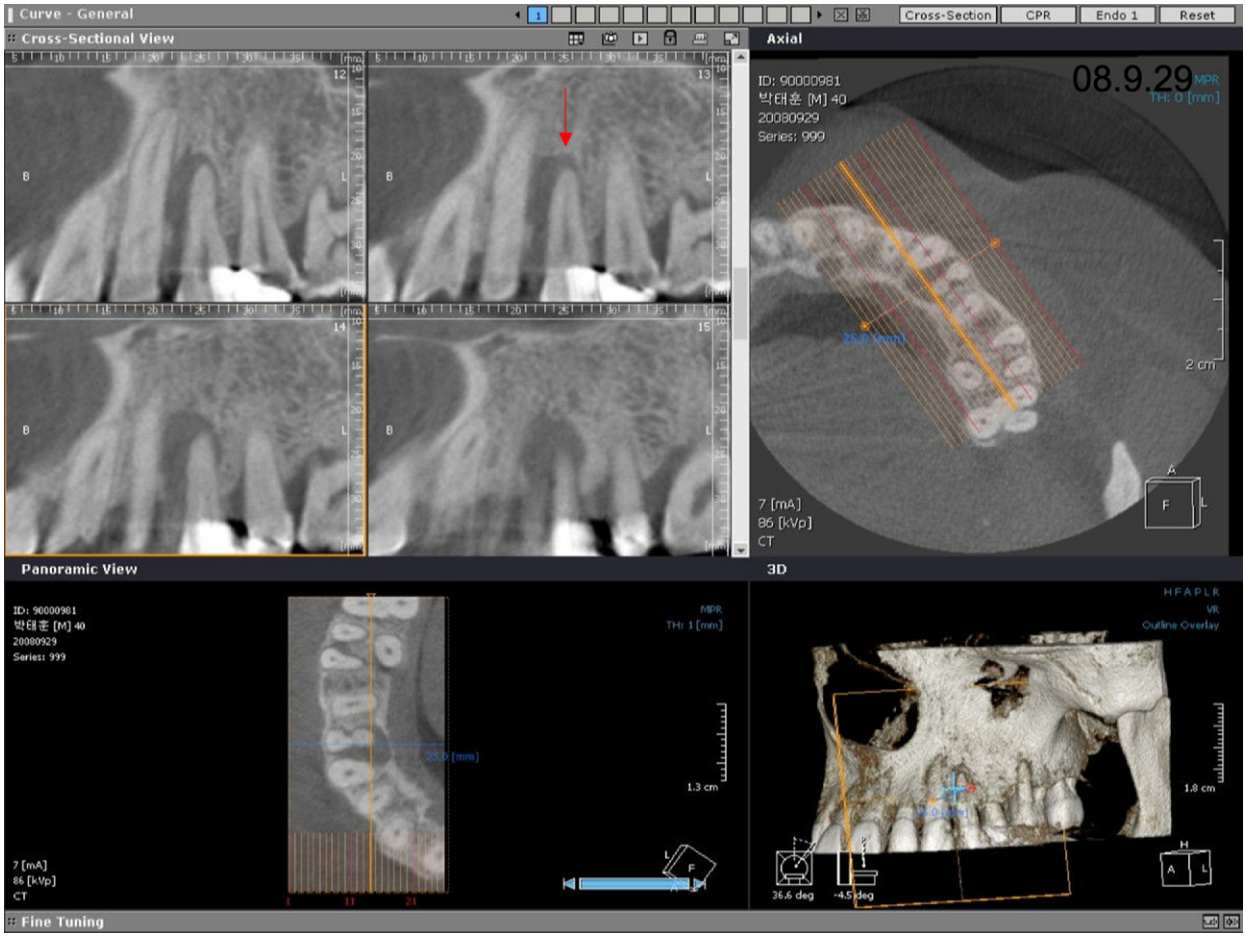

Fig. 3. Computerized tomography image at initial diagnosis. An abscess at the root apex of the left maxillary first premolar is shown.

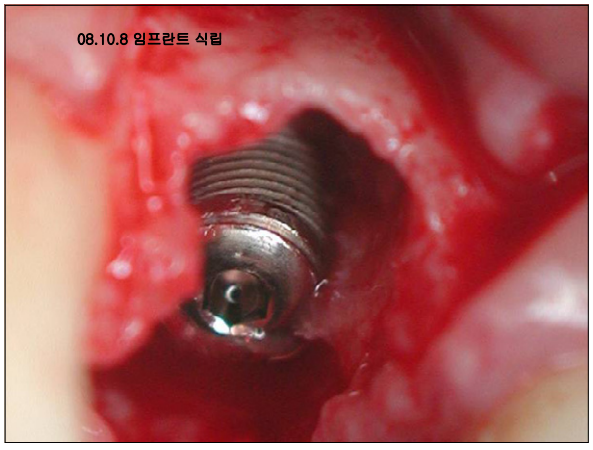

Fig. 4. Appearance after implant placement. A bone defect can be seen in the vicinity of the implant.

weeks, the powder was used for guided bone regeneration during implant placement at the same site (Fig. 4). After 3 months, a second surgery was performed and a temporary fixture was installed. Five months after the bone graft, tissue samples were obtained with the patient's consent (Figs. 5 and 6).

In a total of 6 patients, guided bone regeneration surgery was performed at the time of implant placement, and tissue samples were harvested at the time of the second surgery with the patient's consent. The specimens were fixed in 10% formalin for 24 hours and decalcified in Calci-Clear Rapid (National Diagnostics, Atlanta, GA) for 12 hours. The tissues were rinsed in running water, treated with a Hypercentre XP tissue processor (Shandon, U.K.), embedded in paraffin, cut to a thickness of 4-5 μm, and stained with hematoxylin and eosin.

The prepared specimens were observed via light microscopy, and images were captured using a MagnaFire digital camera system (Optronics, Goleta, CA). The region of interest was measured and analyzed to determine the density of new bone and the proportions of woven and lamellar bone and residual implant material using the Visus Image Analysis System (Image & Microscope Technology, Daejeon, Korea).

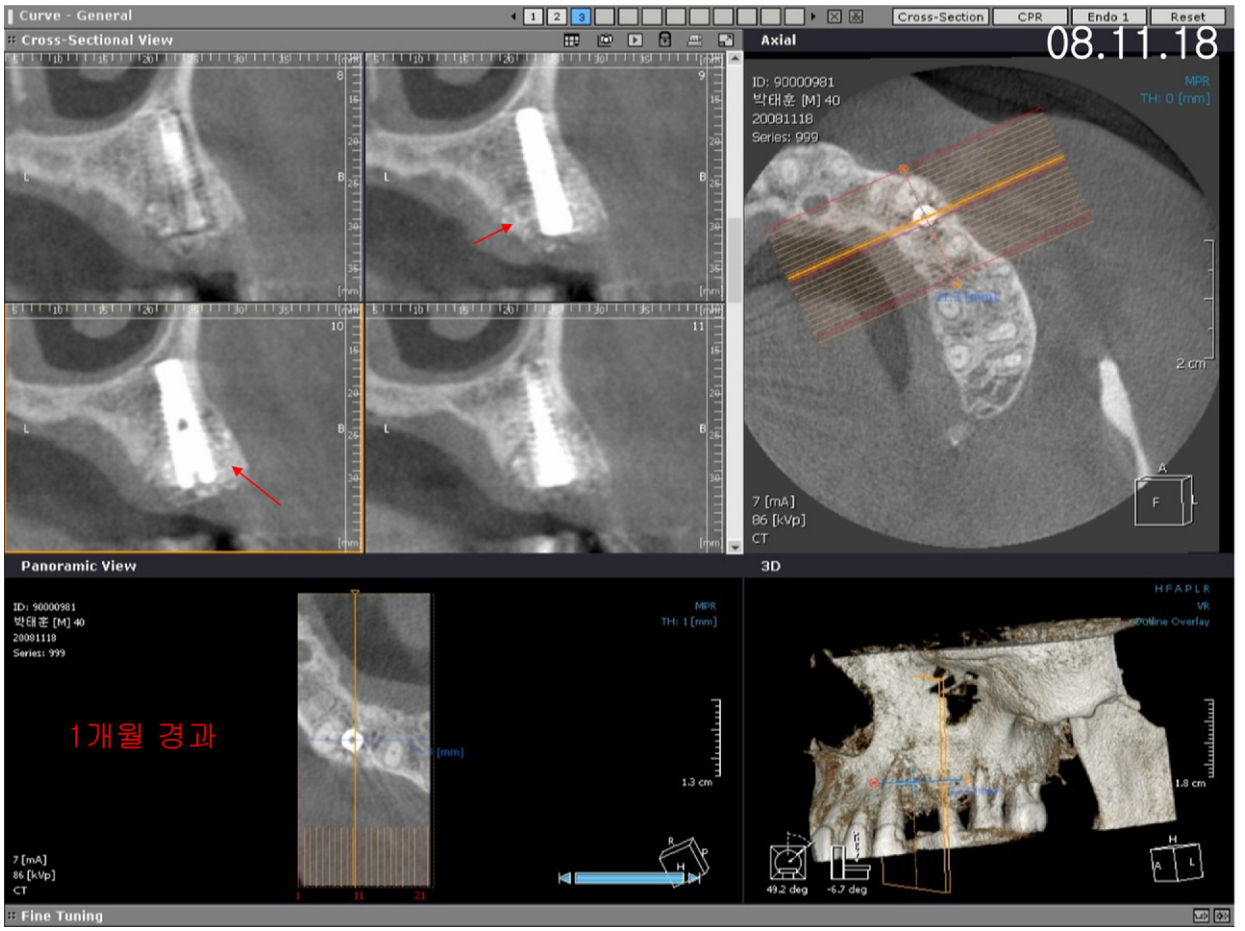

Fig. 5. Computerized tomography image 5 weeks after implant placement. Excellent bone remodeling as a result of bone grafting with AutoBT was observed.

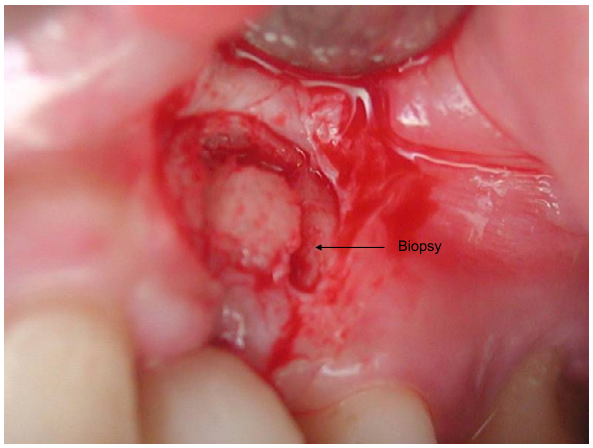

Fig. 6. Five months after the bone graft, tissue samples were obtained. A temporary prosthesis was fabricated 3 months after implant placement.

RESULTS

Analysis of inorganic components

For the analysis of mineral components, X-ray diffraction analysis (XRD) was performed separately on the upper crown portion and the lower root portion. In all areas of the tooth, the primary pattern detected was HA, and the presence of small amounts of β-tricalcium phosphate (β-TCP), amorphous calcium phosphate (ACP), and octacalcium phosphate (OCP) was 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 (Fig. 7).

SEM

After soft tissue removal, the surface of the crushed enamel particles was examined via SEM. This analysis revealed a surface consisting of sharp features, which we attributed to the composition of enamel, which contains HA, a ceramic material with a high degree of hardness. We assumed that, during the crushing process, the destruction of elasticity rather than plasticity occurred (Fig. 8). The enamel surface was examined under high magnification, and we observed that the structure of the particles appeared to have direction,

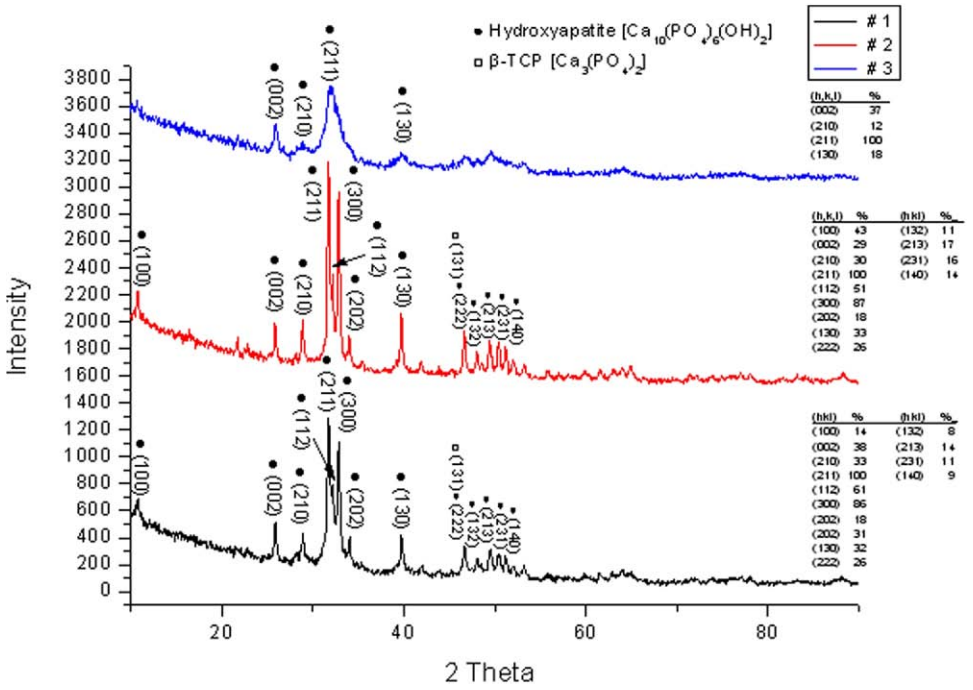

Fig. 7. X-Ray diffraction patterns of powdered human teeth (*#1*, whole tooth; *#2*, root portion; *#3*, crown portion).

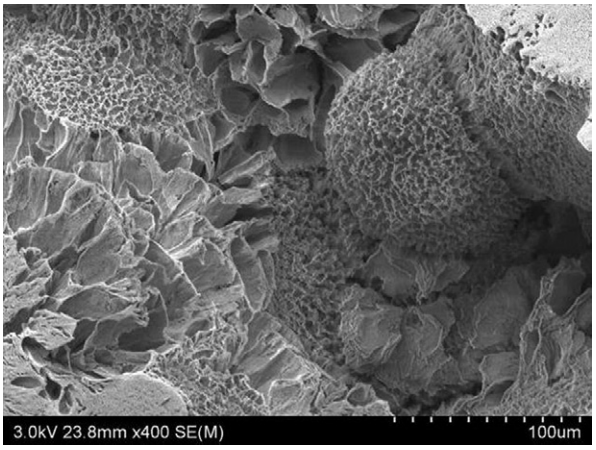

Fig. 8. Scanning electron micrograph of the ground enamel powder surface after removal of soft tissue (×400).

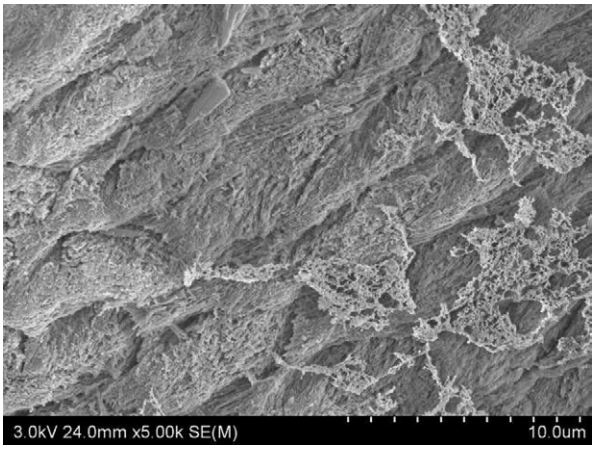

Fig. 9. High-magnification scanning electron microscopy image of ground enamel crystallites after removal of soft tissue (×5,000).

and the porous surface crystals were thought to be HA mineral derived from material close to the enamel during the removal of soft tissues (Fig. 9). In the decalcified enamel area, dentinal tubules and a dense collagen matrix were observed, and the collagen matrix was well exposed in the vicinity of the dentinal tubules (Fig. 10).

Histology and histomorphometric analysis

After 3 months, we observed that most AutoBT underwent resorption, and excellent bone healing and bone remodeling, occurring as a result of osteoinduction and osteoconduction, were observed (Figs. 11-13). In the histomorphometric analysis of the samples collected from 6 patients during the 3-6-month healing period, new bone formation was detected in 46%-87% of the area of interest, and excellent bony remodeling was achieved (Table I). In the samples collected after 3 months, a relatively large amount of the AutoBT was observed and new bone had formed around the grafting

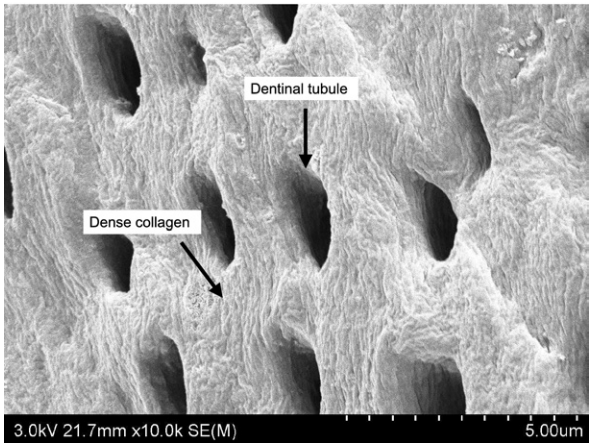

Fig. 10. Scanning electron micrograph of the dentin surface after demineralization (×10,000).

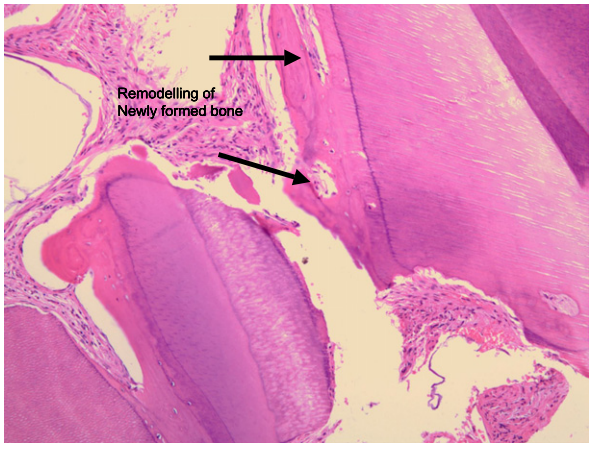

Fig. 11. Histopathologic findings. Newly formed bone demonstrating the occurrence of remodeling was identified around the implanted powder. Remodeling of the tooth elements was noted at bone-implant interfaces (H&E staining, ×40).

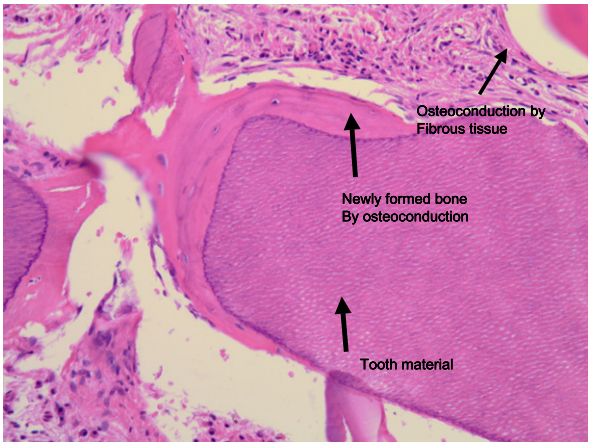

Fig. 12. High-magnification image of new bone formation around the tooth elements. Marginal scalloping of the implant chip suggested that remodeling was occurring at the new bone–implant chip interface (H&E staining, ×100).

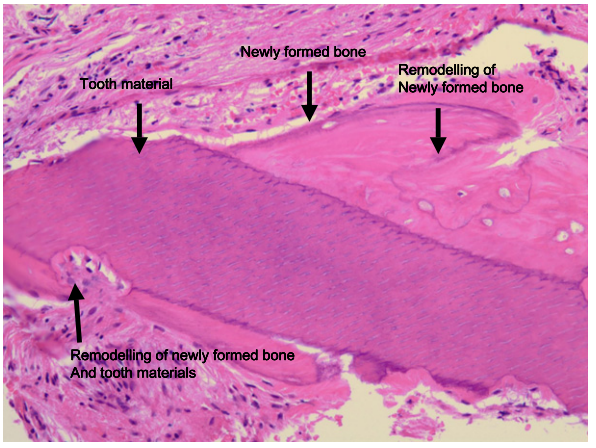

Fig. 13. Newly formed bone and tooth materials showing remodeling were identified around the implant chip and at the periphery of the implant chip, respectively (H&E staining, ×100).

material. Over time, the grafting materials underwent gradual resorption, the amount and shape of the material were reduced and became less visible, and the volume of new bone increased and became interconnected with the surrounding bone, thus forming a more stable structure. After 6 months, the new bone had undergone trabecular bone formation and most of the grafting material had been resorbed. Only a few small pieces were detected around the area of active bone formation.

DISCUSSION

Our long-term efforts to develop bone grafting materials that incorporate allogeneic human teeth and the teeth of animals provided evidence that the inorganic components of teeth were similar to those of alveolar bone. After conducting exhaustive studies, we developed a sufficient basis for the use of extracted teeth as bone grafting material.[2-25] Previous studies established that the removal of organic materials was necessary to abolish infection risk factors when using the teeth of unrelated individuals or animals for grafting; however, bone grafting materials that incorporate both preserved inorganic and organic materials result in rapid alveolar bone remodeling and thus a better prognosis. The AutoBT bone grafting material described here contains

Table I. Histomorphometric findings

Patient	*Age (yrs)/gender*	*Site*	*Healing period*	*WB:LB:IM ratio*	*New bone-forming area (%)*
1	40/M	#24	3	43:11:46	74
2	28/F	#17	4	85:14:1	87
3	47/F	#17	6	56:39:5	46
4	50/M	#24	5	84:12:4	73
5	43/F	#36	3	51:1:48	52
6	61/M	#25-27	6	65:0:35	68

WB, Woven bone; *LB,* lamellar bone; *IM,* residual implant material.

both inorganic and organic material, and the major component of the inorganic material contains 4 types of calcium phosphate. In the analysis of inorganic components performed in our study, HA, TCP, ACP, and OCP were distributed evenly, and active mineral metabolism was demonstrated; consequently, it could be inferred that the reformation of inorganic and organic materials would occur after the actual grafting. The level of HA crystallization in AutoBT and the amount of HA differed greatly depending on the tooth area; the XRD pattern of the crown portion containing enamel was much stronger than that of the root portion. This is likely because HA mineral, with its excellent crystallization properties, makes up approximately 97% by weight of the crown portion, whereas the root portion is composed of relatively little HA mineral and a greater percentage of other organic materials. These results are consistent with those reported by Xue et al.[28]

The chemical composition of teeth is very similar to that of bone. In the enamel, the total inorganic content is 95%, the organic content is 0.6%, and water makes up 4%. However, in the dentin, the inorganic content is 70%-75%, the organic content is 20%, and water makes up 10%; and in alveolar bone, the inorganic, organic, and water contents are 65%, 25%, and 10%, respectively.[29]

Approximately 90% of the organic material present in the dentin consists of collagen fibers, primarily type I collagen, and these fibers play an important role in calcification. The remaining organic components consist of noncollagenous proteins, carbohydrate, lipid, citrate, lactate, etc.[29] Diverse bone growth factors, including bone morphogenetic protein, are known to be present in the protein fraction. Our SEM analysis of calcified dentin revealed dentinal tubules, which are conjectured to act as a network for diffusing nutrients after grafting. The diameter of the dentinal tubules was reported to be 900-2,500 nm.

Our histologic examination of the AutoBT grafting area revealed that the grafting material was gradually resorbed and replaced with new bone, and the new bone formed a direct union with the remaining AutoBT. The healing process, promoted by osteoconduction and osteoinduction, was observed in all samples, and abundant lamellar bone was observed, confirming that bony remodeling was achieved rapidly. Three months after surgery, the autogenous tooth bone grafting material had induced active new bone formation by osteoinduction and was gradually being resorbed. With time, new bone was remodeled into a more stable bone structure, resulting in noticeable trabecular bone formation after 5 months.

The limitations of our study include a large amount of variation in the time at which we collected tissue samples (3-6 months) and the relatively small number of samples, only 1 or 2 each time from each patient. Therefore, our data set may not be representative of all outcomes, and supplemental studies are required. In the future, we will provide an in-depth analysis of the organic materials of AutoBT, and analyses of the inorganic and organic materials of vital and nonvital teeth, caries, gingival diseases, the tooth component of gingival diseases, etc. Presently, AutoBT is being used for bone grafting in the maxillary sinus, augmentation of the alveolar crest, and other diverse clinical cases, and we plan to report clinical outcomes together with the results of histologic studies in the future.

CONCLUSION

Based on previous studies and an analysis of the inorganic components of AutoBT using SEM and histomorphometric analysis, we concluded that AutoBT underwent gradual resorption and was replaced by new bone of excellent quality through osteoinduction and osteoconduction.

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