Analysis of the healing process in sinus bone grafting using various grafting materials

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Objectives. The purpose of this study was to compare differences in the healing process in the sinus bone grafting using various grafting materials.
Study design. Maxillary sinus bone grafts were divided into 4 groups according to the graft material used: group I, a mixture of autogenous bone and BioOss (Osteohealth Co., Shirley, NY); group II, a mixture of BioOss and Orthoblast II (Greencross; Isotis); group III, BioOss only; and group IV, synthetic bone, Osteon (Genoss, Korea), only. To evaluate the healing status of the graft surgery, bone specimens were collected from the lateral sinus using a 2.0-mm trephine bur at 4 and 6 months after surgery. Histology of the bone specimens was prepared, and the percentage of newly formed bone fraction, lamellar bone/woven bone ratio (LB/WB), and newly formed bone/graft material ratio (NB/GM) were measured to indicate the suitability of the materials and the healing of the grafts.
Results. The LB/WB ratio and NB/GM ratio were markedly increased at 6 months compared with the values at 4 months. It was observed that good bone healing was achieved even for grafts of xenogeneic bone only or synthetic bone only. Cases grafted with a mixture of allogeneic and xenogeneic bone showed no great advantage regarding bone healing.
Conclusion. The results indicated that grafts of xenogeneic or synthetic bone can be effective for sinus bone grafting.
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Dental restoration using maxillary posterior implants is known to be difficult and to have a poor success rate. Because it contains abundant type III and IV bone, and because the edentulous state is prolonged, the absolute osteoid volume is insufficient in many cases, owing to pneumonization of the maxillary sinus. However, with the recent development of implant and bone transplant techniques and improvements in the treatment of implant surfaces, implant placement in the maxillary molar area is now possible where the height of residual alveolar bone is insufficient. Sinus bone grafting has been performed generally and has achieved a reasonable prognosis, although the choice of maxillary sinus bone graft material remains controversial.[1-3]

An ideal maxillary sinus bone grafting material should provide biologic stability, ensure volume maintenance, and allow the occurrence of new bone infiltration and bone remodeling. Over time, bone grafting materials and implants should achieve osteointegration. After the restoration of the upper part of the implant has been completed, there should be no bone loss and the materials should be stable; there should be a predictable success rate.[4]

Various bone grafting materials have been studied for use in maxillary sinus grafts to accelerate the bone healing process and prevent repneumonization of the maxillary sinus after grafting. However, most of these reports are from in vitro studies or animal experiments. To fully assess the healing process, bone grafting materials transplanted in humans must be examined histologically. Such studies are obviously limited for ethical reasons, and research studies demonstrating the superiority of a specific material are hard to find.[5,6]

With the approval of the ethics committee from the Bundang Seoul National University Hospital, the objective of the present study was to compare differences in the healing process in the sinus bone grafting using various grafting materials.

MATERIALS AND METHODS

Surgical methods

Surgery was performed under general anesthesia, intravenous sedation, or local anesthesia. A crestal incision was made, and a full thickness flap was lifted. A bony window was made by removing a circular or oval-shaped piece from the anterior wall of the maxil-

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lary sinus. The removed bony window was wrapped with gauze and stored in saline for later use in bone transplantation.

The maxillary sinus membrane was lifted carefully, and a bone graft was performed. The maxillary sinus bone grafts were divided into 4 groups according to the graft material used: group I, a mixture of autogenous bone and BioOss (Osteohealth Co., Shirley, NY); group II, a mixture of BioOss and Orthoblast II (Greencross; Isotis); group III, BioOss only; and group IV, synthetic bone, Osteon (Genoss, Korea), only. For group I, particulate autogenous bone, harvested from the ramus or mandibular symphysis, was mixed with BioOss at a volume ratio of approximately 26%-50% using a tissue adhesive (Greenplast; Korea). For group II, BioOss and Orthoblast II were mixed at a ratio of 1:2. Groups II, III, and IV were transplanted also using a small amount of autogenous bone harvested from the maxillary sinus bony window and the maxillary tuberosity. The number of each specimen in groups I, II, III, and IV was selected randomly. The number of participants varied per group, because not all of the patients agreed to take part in this study. BioOss with a particle size of 1-2 mm was used. For Osteon hydrated in sterile saline, 50% of the particles were 0.5-1 mm and 50% were 1-2 mm. The graft materials were stabilized with Greenplast and placed in the sinus cavity. Where the residual alveolar bone quantity was sufficient for primary stabilization, immediate placement was performed; otherwise, placement was delayed for 4 months. In all cases, the lateral sinus window was covered by a resorbable collagen membrane (Ossix; ColBar R&D, Ramat Hasharon, Israel) before the primary suture. Patients were placed on antibiotics and analgesics. Oral rinsing with 0.12% chlorhexidine was prescribed. To evaluate the healing status of the graft surgery, bone specimens were collected from the lateral sinus using a 2.0-mm trephine bur at 4 and 6 months after surgery.

Harvesting and preparation of tissue samples

Surgery was performed after the patient provided written informed consent. This research was approved by the Institutional Review Board at Seoul National University, Bundang Hospital, on January 24, 2006. For histologic evaluation, biopsy specimens were sent to the Department of Pathology at Chosun University. The histologist was unaware of the surgery and bone graft material (Table I).

In the delayed placement cases, samples were collected 4 months after maxillary sinus bone grafting, immediately before implant placement. In the cases of immediate implant placement at the time of maxillary sinus bone grafting, samples were collected 6 months after surgery. The samples were harvested from the lateral window using a trephine bur, 2.0 mm in diameter. Collected samples were immediately fixed in 10% formalin solution and transported to the Department of Pathology, Chosun University. Samples were decalcified with Calci-Clear Rapid (National Diagnostics, Atlanta, GA) for 12 h. Decalcified tissues were washed with running water, processed using an automatic tissue machine (Hypercentre XP; Shandon, Cheshire, U.K.), and embedded in paraffin. Sections of 4-5 μm were cut, treated with hematoxylin and eosin staining and Goldner trichrome staining, and examined by light microscopy.

Table I. Summary of the histomorphometric study

Group	*NB, %*	*LB/WB*	*NB/GM*
I			
4 months (n = 5)	41.20 ± 7.19	0.26 ± 0.24	2.31 ± 1.30
6 months (n = 5)	48.80 ± 5.63	1.15 ± 1.77	4.36 ± 2.23
II			
4 months (n = 7)	37.86 ± 10.90	0.18 ± 0.20	1.82 ± 2.46
6 months (n = 8)	46.63 ± 13.70	0.32 ± 0.37	3.73 ± 4.35
III			
4 months (n = 5)	35.60 ± 29.99	0.09 ± 0.08	0.78 ± 0.84
6 months (n = 4)	53.00 ± 17.38	0.30 ± 0.31	2.38 ± 1.96
IV			
4 months (n = 9)	40.59 ± 12.80	0.14 ± 0.15	1.95 ± 1.81
6 months (n = 8)	51.88 ± 12.54	0.45 ± 0.35	7.72 ± 8.80

LB/WB, Lamellar bone/woven bone; NB, newly formed bone; NB/GM, newly formed bone/graft material.

Histomorphometric examination

Photographs of the prepared tissue sections were taken with a MagnaFire digital camera system (Optronics, Goleta, CA). Using the Visus Image Analysis System (Image & Microscope Technology, Daejon, Korea), the percentage density of new bone (bone surface/entire sample surface × 100), the ratio of lamellar bone to woven bone (LB/WB), and the new bone as a percentage of graft material (total surface of corresponding implant material/entire sample surface × 100; NB/GM) were measured in each patient. Differences in these measurements between all groups and between the 4- and 6-month samples were analyzed.

Statistical analysis

The histomorphometric analysis of each group according to time point was performed by the Kruskal-Wallis validation method. P values of $<.05$ were deemed to be statistically significant. Statistical analyses were performed using a statistical software package (SPSS 15.0; SPSS, Chicago, IL).

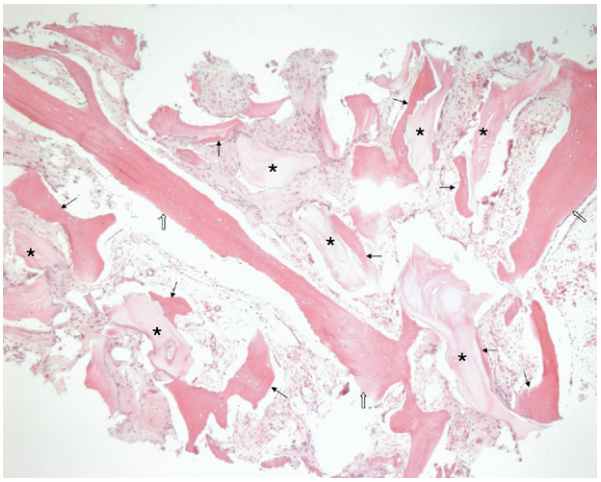

Fig. 1. Histologic finding of group I at 4 months after grafting (H&E stain, ×40). Anastomosing woven bone (*arrows*) was visible around the implant chips (*stars*). Thick trabecular bone (*open arrows*) was also observed.

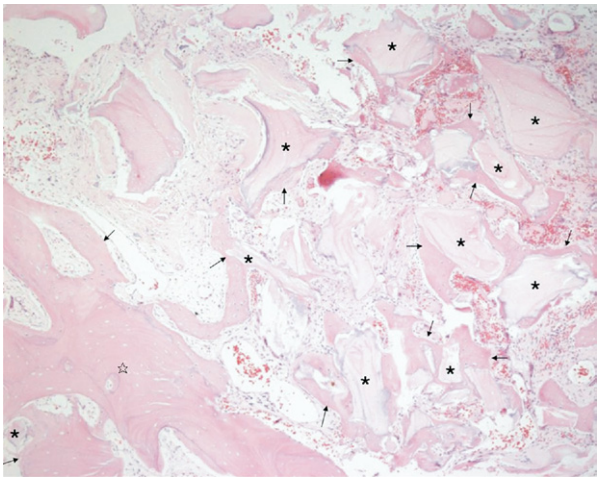

Fig. 2. Histologic finding of group I at 6 months after grafting (H&E stain, ×40). Woven bone (*arrows*) around the implant chips (*stars*) formed trabecular bone (*open star*).

RESULTS

Histopathologic findings

Group I

1. *Four-month observations.* The samples obtained 4 months after surgery exhibited woven bone mutually fused with thick trabecular bone (Fig. 1).
2. *Six-month observations.* In the samples obtained 6 months after surgery, woven bone in the vicinity of the grafting material formed trabecular bone. In comparison with the 4-month samples, the 6-month samples showed a pattern of increased bone density, and lamellar bone was present (Fig. 2).

Group II

1. *Four-month observations.* The woven bone was formed around the implanted material and partly trabecular bone formation was observed. These bones formed a vague anastomosis with the trabecular woven bones formed around the implant (Fig. 3).
2. *Six-month observations.* In comparison with the 4-month samples, the new bone formation was increased with the progress of absorption in implanted material, the new bone formed around the implants was thicker and organized, and anastomosis was formed with new bones in adjacent area to form a solid trabecular bone. These new bones were partially formed by lamellar bones (Fig. 4).

Group III

1. *Four-month observations.* Around the implanted material, newly formed woven bone was formed and partial formation of trabecular bone was observed. Compared with other groups, the new bone formation was weak and the amount of lamellar bone showed the least amount among the groups. Fibrosis between new bones and implanted materials was observed (Fig. 5).
2. *Six-month observations.* In comparison with the 4-month samples, excellent new bone formation was observed. The newly formed woven bone around the implanted materials was much thicker. Because

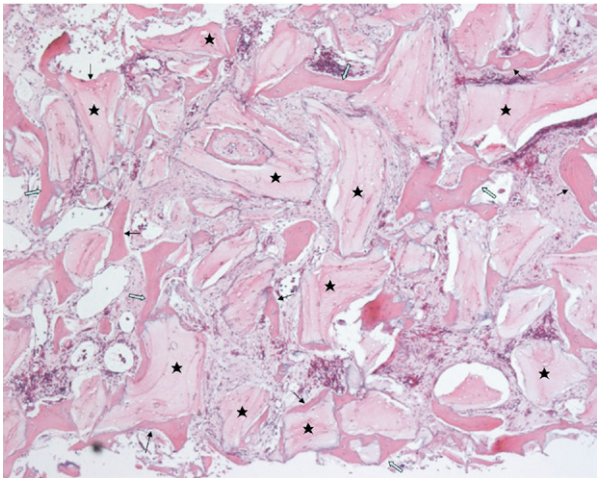

Fig. 3. Histologic finding of group II 4 months after graft (H&E stain, ×40). Woven bone formation (*arrows*) around the implant chips (*stars*) was noted. Trabecular woven bone was anastomosing to the adjacent newly formed woven bone around the implant chips (*open arrows*).

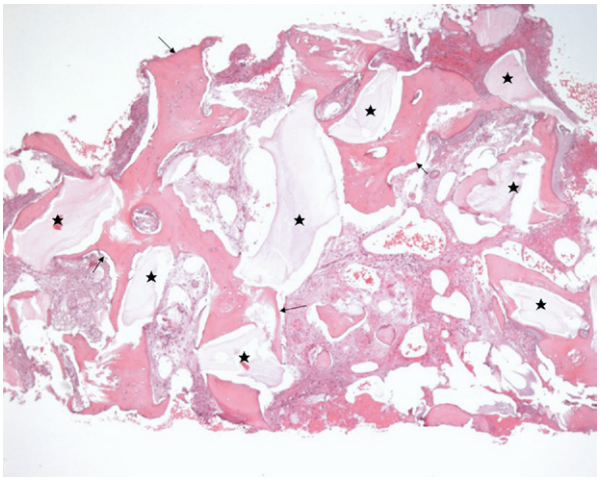

Fig. 4. Histologic finding of group II 6 months after graft (H&E stain, ×40). New bone around the implant chips (*stars*) are forming thick bony trabecular anastomosis (*arrows*). The trabeculae consist of focally lamellar bone.

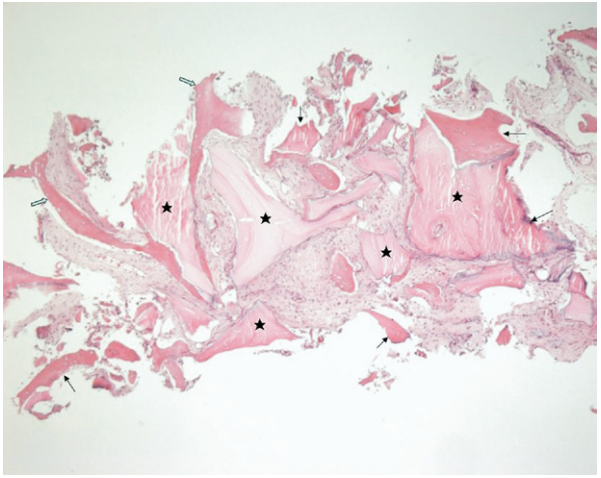

Fig. 5. Histologic finding of group III 4 months after graft (H&E stain, ×40). Newly formed woven bone (*arrows*) was identified around the implant chips (*stars*). The new bone was forming trabeculae (*open arrows*), focally.

there was enough bone around the implants, the anastomosis was formed with surrounding objects to form a solid and stable network. With the progression of implant absorption, the new bone formation was increased and the ratio of implanted material was decreased (Fig. 6).

Group IV

1. *Four-month observations.* At low magnification, the 4-month samples revealed a loose arrangement of developing trabecular bone. The trabecular bone consisted of a relatively thin woven bone with foci of lamellar bone; the intervening stroma showed varying degrees of fibrosis, with infiltrations of chronic inflammatory cells. Higher magnifications demonstrated the newly formed trabecular bone anastomosing hypo- or unmineralized osteoid around the resorbing implant material. Osteoblastic proliferation was identified around the trabecular bone and in the osteoid (Fig. 7).
2. *Six-month observations.* The 6-month samples at low magnification showed more thickened and anastomosing trabecular bone, with resorbing implant material. Compared with the other groups, these samples revealed remarkably thickened bony trabeculae with prominent lamellar bone formation. Higher magnifications revealed well organized thickened anastomosing lamellar bone around the resorbing implant material and variable intervening stromal fibrosis (Fig. 8).

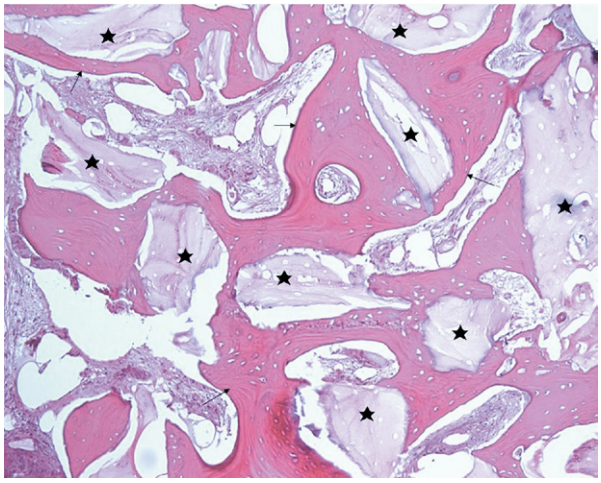

Fig. 6. Histologic finding of group III 6 months after graft (H&E stain, ×40). Trabecular woven bone (*arrows*) around the implant chips (*stars*) was anastomosing. The trabeculae are thicker and the implant chips are resorbing.

Histomorphometric findings

There were no statistically significant differences in the bone density, LB/WB ratio, or NB/GM ratio of the 4-month samples or the 6-month samples between any of the groups ($P > .05$; Table I). However, despite the lack of statistical significance, the mean values for the bone density, LB/WB ratio, and NB/GM ratio at 4 months were all highest in group I, which was grafted with a mixture of autogenous and xenogeneic bone, and the values for the LB/WB and NB/GM ratios were lowest in group III, which was transplanted with xeno-

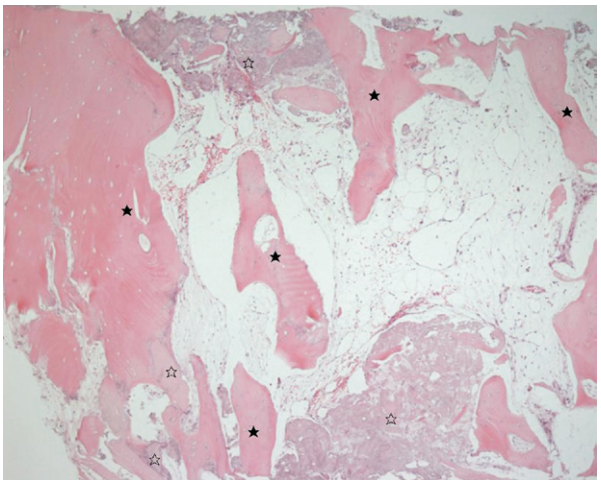

Fig. 7. Histologic finding of group IV 4 months after graft (H&E stain, ×40). Anastomosing trabecular bone (*stars*) consisting of woven bone and lamellar bone was seen, as well as new bone formation around the resorbing implant material (*open stars*).

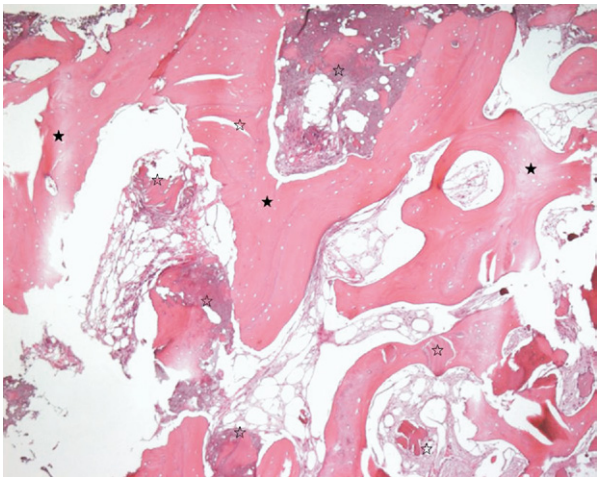

Fig. 8. Histologic finding of group IV 6 months after graft (H&E stain, ×40). Thickened focally lamellar trabecular bone (*stars*) was seen around the resorbing implant material (*open stars*).

geneic bone only. In each of the groups, including those transplanted with xenogeneic bone only (group III) and synthetic bone only (group IV), the values for the bone density, LB/WB ratio, and NB/GM ratio were markedly increased at 6 months compared with the values in the group at 4 months.

DISCUSSION

Currently, maxillary sinus bone grafting is predictable and considered to be a safe procedure. The maxillary sinus is a type of "contained-type defect," and most biocompatible bone grafting materials can be used successfully. With time, maxillary sinus bone grafting materials may undergo resorption.[7] Hatano et al.[8] reported that, in the initial 2-3 years, the material may undergo pneumonization; to avoid this, grafting materials should be nonabsorbable or only slowly absorbed. It was recently demonstrated that the healing pattern in maxillary sinus bone grafting did not differ greatly among a variety of grafting materials; autogenous, allogeneic, xenogeneic, and synthetic bone could all be used safely, and the appropriate material could be chosen according to the preference of the surgeon. However, the inclusion of an appropriate amount of autogenous bone in the grafting material has been reported to substantially shorten the healing process, owing to greater bone formation and osteoinduction.[9] An ideal maxillary sinus bone grafting material should induce the formation of a high ratio of vital bone. Additionally, it should prevent repneumonization after resorption of the graft material. Several investigators[10-12] have strongly recommended a mixture of autogenous bone and xenogeneic or synthetic bone, and various types of bone grafting materials have shown 14%-44% vital bone content.[10,13] In the present study, the 4 groups showed 35%-41% vital bone content at 4 months and 46%-53% at 6 months.

In 1988, Wood and Moore[14] performed maxillary sinus bone grafting using autogenous bone harvested from the ascending ramus and the coronoid process. The advantages of using autogenous bone for grafting include faster reformation of blood vessels, bone formation immediately after grafting (phase I bone formation), and the availability of various types of bone pieces, such as particles, plates, and blocks, for harvesting. Additionally, autogenous bone is nonantigenic and highly reliability. Maxillary sinus bone grafting has been performed using only autogenous bone in many past cases. Nishibori et al.[15] reported that those cases of maxillary sinus bone grafting that used autogenous bone had the most desirable outcomes. On the other hand, some investigators[16,17] have reported that autogenous cortical bone showed unpredictable reactions after grafting and did not provide the most desirable results for the long-term survival of implants. Others have reported that autogenous bone can be absorbed over a long time period and that continuous repneumonization of the maxillary sinus can occur, threatening the long-term survival of the implant. We also suspected that the resorption volume would be substantial and sinus pneumonization would develop if only autogenous bone were used. Thus, autogenous bone was not used as the only grafting material for maxillary sinus bone grafting in this study, and there was no

autogenous bone–only grafting group included as a control group.

With demineralized freeze-dried bone allograft (DFDBA), the resorption stage is omitted during the bone healing process, making more rapid bone formation possible; nonetheless, the physical strength of the new bone is weak. Therefore, if only demineralized bone were used for procedures in areas requiring physical strength, such as maxillary sinus bone grafting or alveolar crest augmentation, the grafting material may be completely absorbed before complete bone healing.[18] For these reasons, we do not use DFDBA alone, and it was not included in this study.

Kim et al.[19] have reported animal studies on bone formation with grafting materials containing demineralized bone matrix (Orthoblast II). At 3-6 weeks after the graft, the bone density was higher in the demineralized bone matrix (DBM) group than in the autogenous bone group. Nevertheless, after 8 weeks, the bone density in the autogenous bone group was 54.3% and that in the DBM group was 45.1%. Thus, DBM had increased the density of new bone only during the early period. Mardas et al.[20] reported that DBM did not increase overall new bone volume but increased bone density, in animal experiments; Stentz et al.[21] and Liljensten et al.[22] reported similar results. In our previous case study,[23] we transplanted a mixture of a type of DFDBA (Orthoblast II) and allogeneic bone (BioOss). This mixture was also used in a group of cases in the present study, because we thought that each component might compensate for the shortcomings of the other, given the osteoinduction properties of bone morphogenic proteins and the osteoconduction properties of allogeneic bone. In this way, new type 1 or 2 bone formation might be achieved, with the possibility of shortening the healing and bone remodeling periods. However, no great difference in terms of bone healing was detected between this group and any other experimental group in the present study.

For xenogeneic bones, the treatment of antigens is very important; thus, in addition to decalcification or freeze-drying, additional treatment processes to remove antigens are required. The effects of xenogeneic grafts have been examined in various surgeries such as fresh extraction, the local defect area of the alveolar crest, and maxillary bone grafts, showing that appropriately treated xenogeneic bone is biocompatible and fuses well with recipient areas; postsurgical complications were infrequent. It has also been reported that the survival of implants placed together with maxillary sinus bone grafting using xenogeneic or synthetic bone was superior to using autogenous bone. Even if a substantial amount of graft material were not absorbed, it has not been proven that residual implant material would impede the bone fusion of implants. In fact, bone density was increased noticeably and may increase the long-term survival of implants.[24,25] It was similarly observed in the present study that bone density increased with time in groups 3 and 4. Maiorana et al.[26] used alloplastic bone, hydroxyapatite (HA), and collagen, or xenogeneic bone and Bio-Oss for sinus grafts and followed patients for 4 years; they reported a 97% survival rate. They proposed that these materials, as nonabsorbable materials, could provide appropriate initial stability and be useful graft materials.

Anorganic bovine bone revealed no more than 0.5-1 mm absorption after 4 years and showed an opaque image on X-ray, suggesting that it too is a good material for sinus grafting. Yildirim et al.[27] performed maxillary sinus bone grafting using anorganic bovine bone, harvested samples after 6 months, and conducted a histomorphometric analysis. They found that the samples were 14.7% new bone, 29.7% residual xenogeneic bone grafting material, and 56.0% soft tissues. Ozyuvaci et al.[28] conducted histologic tests 6-8 months after performing maxillary sinus bone grafting using BioOss or β-tricalcium phosphate (β-TCP) and reported 45%-50% new trabeculae and 25%-30% residual grafting material in the BioOss group and 50%-55% new trabeculae and 15%-20% residual graft material in the β-TCP group. Hürzeler et al.[29] evaluated the clinical and histologic results of sinus grafting using porous hydroxyapatite. They used a 1:1 mixture of BioOss and Interpore 200, a 1:3 mixture of autogenous bone from the iliac crest and Interpore 200, and a 1:1 mixture of autogenous bone from the mandibular chin bone and Interpore 200. They placed 340 implants (235 immediate placements and 105 delayed placements) in 133 patients and evaluated the patients 5 years after metal ceramic prosthesis placement. All (100%) of the placed implants showed successful osseointegration; only 4 implants were removed for failure of the prosthesis, indicating a success rate of 98.8%. Additionally, they confirmed that the load on the implant increased osseointegration of the implant surface and that the porous HA increased bone formation and bone-implant contact. At 4-5 months after performing sinus grafting using porous HA in 4 patients, Smiler and Holmes[30] prepared specimens for histologic analysis and reported an average of 23% new bone, 45% connective tissue, and 32% porous HA. In a similar study, Moy et al.[31] reported similar results: 20% new bone, 47% connective tissue, and 33% graft materials. In the present study, approximately 53% new bone was seen in the BioOss-only group after 6 months and approximately 52% new bone trabeculae in the Osteon-only group; these results are similar to those of Ozyuvaci et al.[28] We also used a new alloplastic material developed in

South Korea, the main components of which are HA and β-TCP, for sinus grafting and showed favorable healing 6 months after grafting. In addition, the histologic appearance of the bone healing observed in this study was as good as that in earlier studies. However, it is difficult to explain the healing process with alloplastic bone alone, because of the insufficient sample quantity and the mixed use of a small amount of autogenous bone in this study. Furthermore, owing to the shortness of the histologic observation period, we cannot predict the future of the graft material and cannot rule out the possibility of absorption of the sinus graft.

Autogenous, allogeneic, xenogeneic, and synthetic bone each have their own advantages and shortcomings; it has not been determined that a specific material is noticeably superior. Thus, in the clinic, to stimulate bone healing while minimizing repneumonization of the maxillary sinus, there is a tendency to use autogenous bone together with other bone grafting materials that are absorbed slowly. Hatano et al.[8] reported that repneumonization is possible during the initial 2-3 years, and to avoid it, nonabsorbable or slowly absorbable materials should be used. Several investigators have recommended the use of a mixture of autogenous and synthetic bone.

This histologic study on bone grafting materials was conducted in humans, and unlike in animal studies, limits exist in assigning control groups. Given that resorption is substantial and the risk of progression to repneumonization is high in grafting of autogenous bone only, for ethical reasons we do not have a true control group. The number of samples was small, and therefore in the histomorphometric analysis, statistically significant differences in the ability to form bone could not be detected among the 4 groups. For maxillary sinus bone grafting in cases in which variables such as the presence of perforations of the maxillary sinus mucosa and the height of residual bone are not concerns, a good healing process can result regardless of the type of bone grafting material used. It was further observed that good bone healing was achieved even for grafts of xenogeneic bone only or synthetic bone only. Cases grafted with a mixture of allogeneic and xenogeneic bone showed no great advantage regarding bone healing. This is an encouraging result, considering the burden of additional surgery for autogenous bone grafts and the high cost of commercially available allogeneic bone. This study was limited by its preliminary nature and small sample number. As a result, we plan to compare differences in maxillary sinus bone grafting according to grafting materials through additional studies in the future.

Because the purpose of this study was to evaluate the healing stage at 4 and 6 months after sinus bone grafting, the success rate and failure rate of the implant was not included, because many of the cases were <1 year after prosthetic treatment. Future reports will include data >1 year after prosthetic treatments, including resorption volume of the maxillary sinus bone grafting material, the implant marginal bone resorption, and short-term success and survival rate. However, based on the observations from the present study, it was concluded that grafts of xenogeneic or synthetic bone can be successfully used for use in sinus bone grafting.

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