Evaluation of Bone Formation After Grafting With Deproteinized Bovine Bone and Mineralized Allogenic Bone

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Purpose: *The purpose of this study was to evaluate the ability of new bone formation of deproteinized bovine bone (Bio-Oss) and mineralized allogenic bone (Tutoplast).*

Materials and Methods: *Sixty rats were divided into control and experimental groups (groups 1 and 2): control group, unfilled control; group 1, Bio-Oss; group 2, Tutoplast, respectively. The animals were killed after 6 and 12 weeks, and newly formed bone was analyzed histomorphometrically.*

Results: *In the control group, some new bone formed in the rim of the defect area. In the group 1, newly formed bone was thinner than the adjacent normal bone, and Bio-Oss particles were observed. In the group 2, showed a pattern of gradual fusion with adjacent bone, as well as particles in some areas, similar to the Bio-Oss–treated group. In the 12-week groups, the amount of new bone formation was significantly higher in the experimental groups than in the control group, and it was significantly higher in group 2 than in group 1.*

Conclusion: *Although Tutoplast and Bio-Oss graft materials seem to be useful for bone grafts, Tutoplast showed more active new bone formation than Bio-Oss. (Implant Dent 2015;24:101–105)*

Key Words: guided bone regeneration, osteoconduction, osteoinduction

Autogenous bone is recognized as the gold standard,[1–3] but it has shortcomings due to additional surgeries at the intraoral and extraoral donor sites, which consequently results in pain in the donor site, development of complications, prolongation of operation time, and a limitation in the amount of bone that can be harvested.[4,5] To overcome such disadvantages, allobone, xenobone, or alloplastic bones have been developed.[6,7]

Bio-Oss (Geistlich Pharma AG, Wolhusen, Switzerland) is a bovine-originated, anorganic, bone graft material prepared from cows by heating at relatively low temperature (300°C) to remove organic substances using alkaline chemicals and by sterilization with dry heat. Proteins are removed by these processes, pathogens are absent, and this type of bone does not induce an immune reaction, eliminating the possibility of an infection.[6] Additionally, the natural bone structure is maintained, and it is a multiporous structure similar to human bones (75%). Bio-Oss facilitates the formation of blood vessels and migration of osteoblasts, and new bone is formed by osteoconduction in a bone graft.[6,8–10]

Tutoplast (Tutogen Medical GmbH, Neunkirchen, Germany) is allogenic bone obtained from human cadavers that is manufactured by the following special tutoplast processing technique according to the standards of American Association of Tissue Banks[11]: delipidization using acetone and ultrasound, osmotic treatment using distilled water and a saline bath, oxidative treatment using a hydrogen peroxide solution, serial dehydration, and gamma irradiation (17.8 Gy). This is a solvent preserved method, and because it removes water, the mineral matrix is preserved better than freeze-dried allogenic bone. After processing, it is a non-demineralized allogenic graft material that contains all of the minerals and collagen matrix structure of the human skeleton.[11–13]

The purpose of this study was to evaluate the osteoconductivity of 2 kinds of bone substitutes (deproteinized bovine bone [Bio-Oss] or mineralized allogenic bone [Tutoplast]).

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Fig. 1. Histopathological findings of the 12-week control group. A small amount of new bone (arrows) was found in the rim of the defect area (hematoxylin and eosin ×50).

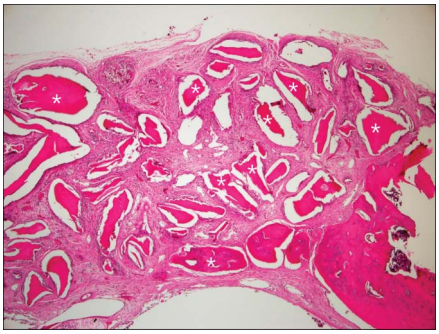

Fig. 4. Histopathological findings of the 6-week group 2. Transplanted with Tutoplast (white asterisks), a fusion pattern with adjacent bones having little inflammation was observed (hematoxylin and eosin ×50).

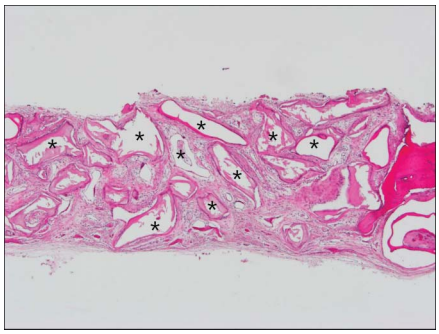

Fig. 2. Histopathological findings of the 6-week group 1. The graft material particles (black asterisks) were not fused with adjacent bone and were separated from new bone (hematoxylin and eosin ×50).

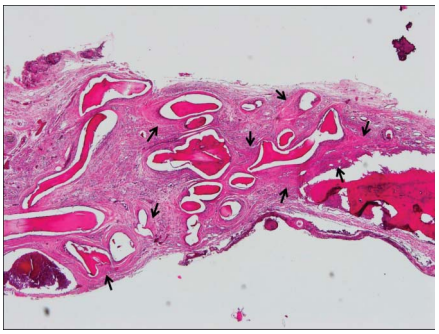

Fig. 5. Histopathological findings of the 12-week group 2. More bone formation (arrows) than the 6-week experimental group 2, and some areas could not be distinguished from adjacent bone (hematoxylin and eosin ×50).

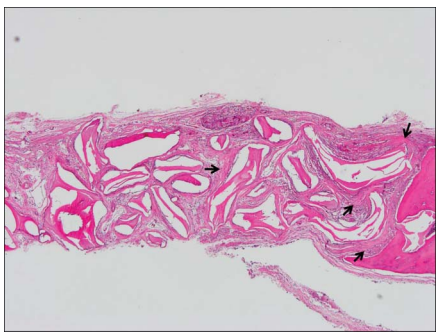

Fig. 3. Histopathological findings of the 12-week group 1. New bone (arrows) could be distinguished from adjacent bone, but areas remained that could not be distinguished (hematoxylin and eosin ×50).

MATERIALS AND METHODS

Animals

Experiments were conducted on 60 male 9-week-old Sprague-Dawley rats weighing 200 to 300 g. Before experimentation, the protocol was evaluated and approved by the Animal Research Committee of Chosun University to ensure that the policies, standards and guidelines for the proper use, care, handling, and treatment of animals were observed. They were maintained under identical conditions, and all animals were healthy.

Materials

Bio-Oss was purchased from Geistlich Pharma AG. It is a bovine-originated demineralized omentum graft material, and the 0.25- to 1-mm particle size was used. Tutoplast was obtained from Tutogen Medical GmbH. It is a graft material originating from humans prepared by a solvent preservation method, and the 0.25- to 1-mm particle size was used.

Experimental Groups

Bio-Oss (group 1) or Tutoplast (group 2) was transplanted into a created cranial defect area in 20 rats; 10 animals from each group were killed at 6 weeks, and the remaining 10 animals at 12 weeks. The control group consisted of animals that did not receive a bone graft. The animals were killed after 6 and 12 weeks.

Formation of a Cranial Defect Area and Bone Grafting

The animals were injected intramuscularly with 2.2 mg of a 2% Rampun injection solution (Bayer Korea Ltd., Seoul, Korea) and 0.18 mg ketamine chloride injection solution (Yoohan Yanghang, Seoul, Korea). Infiltration anesthesia with lidocaine chloride containing epinephrine diluted to 1:100,000 (Yoohan Yanghang) was applied to the cranial bone defect area to suppress local hemorrhage and pain. The surgical area was sterilized with potadine, and the cranial area was removed. An incision was made along the midline of the head to expose the skull. A hole 8 mm in diameter was then drilled in the skull, removing the entire layer of the skull using a 1/4 round bur. After grafting, the peristeum was sutured, and layer-to-layer suturing was performed with absorbable sutures. After the surgery, 6 mg gentamicin (Joongwei Phamaceutical, Seoul, Korea) was injected intramuscularly to prevent infection.

Sample Preparation

Animals were anesthetized as described above and killed in each experimental group. Then, the defect area including the upper periosteum, the scalp, and adjacent healthy bone was resected, fixed in 10% neutral formalin, and decalcified by acid immersion (Calci-Clear Rapid; National Diagnostics, Atlanta, GA) for approximately 4 hours. In the middle of the bone defect area, 3 sections of 3-mm thickness were harvested consecutively, washed, and embedded in paraffin using an automatic tissue preparation instrument (Hypercenter XP,

Shandon, United Kingdom). Paraffin blocks 4 to 5 μm in thickness were prepared, attached to glass slides, maintained at 68°C on a heat plate for longer than 1 hour, and stained with hematoxylin-eosin or Masson's trichrome stains. The prepared specimens were observed under microscopy, and images were captured with the MagnaFire digital camera system (Optronics, Goleta, CA). The region of interest was measured and analyzed for the amount of new bone formation using the Visus Image Analysis System (Image and Microscope Technology, Daejeon, Korea).

Statistical Analysis

The area of bone formed from the edge of defect area to the center was measured (n = 3 per sample), and an analysis of variance was performed. Scheffe test was performed to assess significance among the experimental groups. $P < 0.05$ was considered statistically significant.

Results

Histological Results

A small amount of new bone in the rim of the defect area could be detected in the control group cases, but infiltration by inflammatory cells was limited. However, a new bone formation pattern with fibrous connective tissue, but no bone formation, was revealed in the center of the defect area. More new bone formed in the 12-week group than in the 6-week group, but the bone was thinner than normal bone, with fewer lacunae within the bones (Fig. 1).

In group 1, the Bio-Oss that filled the defect area was maintained well without invasion of soft tissue. Nonetheless, bone was not formed, a small amount of fibrous connective tissue and Bio-Oss particles without resorption were observed. The area where new bone formed maintained a thickness that was thinner than adjacent normal bone, and more bone formed in the 12-week group than in the 6-week group. In the 6-week group, an area occurred in which the graft material particles were not fused with adjacent bone and were separated from new bone (Fig. 2). In the 12-week group, new bone could be distinguished from adjacent bone, but areas remained that could not be distinguished. Nevertheless, most particles were not resorbing (Fig. 3).

In the 6-week experimental group transplanted with Tutoplast, a fusion pattern with adjacent bones having little inflammation was observed. Although more bone formed in the defect area than in the Bio-Oss graft group, some areas showed a small amount of fibrous connective tissue. Tutoplast particles were observed in some areas, but less of a fusion pattern with adjacent bone occurred when compared with the Bio-Oss graft group (Fig. 4). The 12-week group had more bone formation than the 6-week group, and some areas could not be distinguished from adjacent bone (Fig. 5).

Active osteoblast activity and active formation of new bones were detected around the defect area in all groups, but the amount of new bone formation was greater in the experimental groups than the control group. The new bone was markedly thinner, with abundant fibrous connective tissues. Group 2 had more active formation of new bone compared with group 1.

Histomorphometric Analysis

Among the 6-week groups, the amount of new bone formation in groups 1 and 2 were 0.43 ± 0.04 mm^2 and 0.39 ± 0.03 mm^2, respectively, which were significantly higher than in the control group (0.22 ± 0.03 mm^2). In the 12-week groups, the amount of new bone formation in experimental groups 1, 2, and the control were 0.55 ± 0.06 mm^2, 0.61 ± 0.16 mm^2, and 0.29 ± 0.02 mm^2, respectively. The new bone formation was significantly higher in the experimental groups than in the control group, and it was significantly higher in group 2 than in group 1 ($P = 0.001$) (Table 1).

Table 1. Mean Amounts of New Bone Formation in Control, Groups 1 and 2 at 6 and 12 Weeks After Placement (in Square Millimeters)

	Mean ± SD		
Time period (wk)	Control	Group 1	Group 2
6	0.22 ± 0.03	0.43 ± 0.04*	0.39 ± 0.03*
12	0.29 ± 0.02	0.55 ± 0.06*	0.61 ± 0.16*†

*Statistically significant difference relative to control ($P < 0.05$).
†Statistically significant difference relative to group 1 ($P < 0.05$).

Discussion

Bone grafting is performed in a bone defect area to reconstruct congenital deformities or to correct injury to the oral and maxillofacial area caused by trauma, facial deformity, tumors, or other diseases, as well as for esthetic and functional recovery. Bone transplants, autogenous bones, allogenic bones, xenogenic bones, and synthetic bone substitutes have all been used as bone graft materials.[14,15]

Antigens are removed in allogenic bones, and heat-treated, frozen, freeze-dried, or irradiated bones are used for an effective bone graft. However, heat treatment and irradiation impair bone formation after the graft, whereas freezing, freeze-drying, or a demineralized freeze-drying method do not to impede bone formation after grafting.[16] Allogenic bone is supplied as frozen, freeze-dried, or as demineralized freeze-dried bone, and demineralized freeze-dried or non-demineralized freeze-dried bone has been used most widely.

Freeze-dried bones have been used in the orthopedic surgery field since the 1950s, and they have been used in the dental area since the 1970s.[17] Demineralized freeze-dried bone allografts (DFDBAs) mediate the differentiation of host undifferentiated mesenchymal cells to osteoblasts and thus form new bone; DFDBAs were used for the first time in 1975 to treat periodontal defects.[18]

Demineralized bone contains bone morphometric proteins (BMPs), but the bone-forming activity of BMPs is suppressed by inorganic substances within bones; BMPs, however, are not removed during the demineralization process, allowing for excellent osteoinduction of new bone.[19]

Tutoplast (Tutogen Medical GmbH) is allobone obtained from the human cadaver, and it is processed through the

special process called Tutoplast processing technique.[18,20]

It is processed through 5 steps following delipidization, osmotic treatment, oxidative treatment, dehydration, and the gamma irradiation (17.8 Gy). And the mineralization substrate is well preserved because the moisture is removed by solvent preserved method. It is mineralized allobone has the mineral of the human and collagen substrate.[18]

Bio-Oss (Geistlich Pharma AG) is a bovine bone derivate that undergoes a low heat (300°C) chemical extraction process by which all organic components are removed, but maintains the natural architecture of bone. However, proteins were detected in Tutoplast (bovine), Bio-Oss, and tibia samples treated at the similar condition for Bio-Oss deproteinization through systemic review currently.[21] The vascularization and migration of osteoblasts occurs readily because it has a multiporous structure similar to human bones. So although osteoinductive ability is absent, it has very high osteoconduction properties.[8] And Liu et al[22] reported that the mean rates of mineralization of the bony tissue around the implant in Bio-Oss graft group were significantly higher than non treated group after extraction, graft of Bio-Oss into the extraction sockets can promote osseointegration after delayed implantation. Schmitt et al[23] performed comparative study of Bio-Oss and autologous bone plus Bio-Oss, they reported that there was no significant difference in newly formed bone. According to a study by Merkx et al,[24] inflammation was observed after a graft in rats, and after 8 weeks, no infiltration of inflammatory cells occurred. Similarly, we observed no infiltration of inflammatory cells in the group grafted with Bio-Oss.

Lee et al[25] compared the osteoconductive effects of deproteinized bovine bone mineral (Bio-Oss) and solvent-dehydrated allograft (Tutoplast) in extracted socket of human. They reported the inflammatory cell infiltration was rare in both materials like our study. But, deproteinized bovine bone mineral induced more new bone deposition in the periphery of the native bone particles than solvent-dehydrated allograft. Tudor et al[26] studied about new bone formation in calvarial defect of pigs using particulated human materials and bovine materials. They reported that the microradiographically measured mineralization rate was 5% to 10% lower than the mineralization rate of autogenous bone grafts, but statistical analysis showed no significant differences after 12 weeks. Our results are slightly different than results of these studies, the new bone formation was significantly higher in Tutoplast graft than in Bio-Oss graft in this study after 12 weeks. However, it cannot conclude Tutoplast is superior than Bio-Oss based on this study has limit in which the sample is small. Bosetti et al compared Bio-Oss and Tutoplast as graft for ridge augmentation. They reported that the bovine-derived bone may be considered a good bone substitute for clinical situations requiring greater time of permanence of the material, whereas human-derived bone will be more suitable for clinical situations required lower time of permanence because the bovine-derived bone showed lowest macrophage and osteoclast activator.[27]

CONCLUSION

A bone defect area was generated in the rat cranium, and Bio-Oss and Tutoplast, which are most widely used in clinics, were transplanted. Using histological and histomorphometric evaluations, the osteoconduction of these 2 bone substitutes was compared, and the following results were obtained.

1. A small amount of new bone formed in the rim of the defect area in the control group; however, bone did not form in the center and fibrous connective tissue was observed.
2. In the group grafted with Bio-Oss, newly formed bone was thinner than the adjacent normal bone, and Bio-Oss particles were observed.
3. The group grafted with Tutoplast showed a pattern of gradual fusion with adjacent bone, and particles were observed similar to the Bio-Oss group.
4. In the 12-week groups, the amount of new bone formation was significantly higher in the experimental groups than in the control group, and Tutoplast group was higher than Bio-Oss group ($P = 0.001$).

DISCLOSURE

The authors claim to have no financial interest, either directly or indirectly, in the products or information listed in the article.

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