A clinical study on bone formation using a demineralized bone matrix and resorbable membrane

Young-Kyun Kim,[a] Su-Gwan Kim, DDS, PhD,[b] Sung-Chul Lim,[c] Hyo-Jung Lee,[d] and Pil-Young Yun,[e] GwangJu City, South Korea
SEOUL NATIONAL UNIVERSITY BUNDANG HOSPITAL AND CHOSUN UNIVERSITY

Objectives. The purpose of this study was to evaluate new bone formation following guided bone regeneration (GBR) using a composite of demineralized cortical and nondemineralized cancellous bone admixed in a poloxamer reverse phase carrier (Orthoblast II) and resorbable collagen membrane (Ossix).
Study design. Fourteen patients (14 specimens) participated in this study from January 2006 to May 2006. In all these 14 patients, bone grafting for the regeneration of dehiscence defects around the implants was required. At the 4- and/or 6-month healing period, a biopsy specimen was obtained by one oral and maxillofacial surgeon. The specimens were fixed, demineralized, embedded, and sectioned by a pathologist, and histomorphometric evaluations were performed using a computer-assisted Visus Image Analysis System.
Results. A high proportion of new bone formation (12.3%-78.7%) was observed during the 4- and/or 6-month healing period. Although histopathologic findings indicated that the grafted materials did not completely resorb, new bone formation and bone remodeling were observed to increase with healing time.
Conclusion. It was concluded from this study that the use of GBR consisting of Orthoblast II and Ossix membranes caused favorable bone formation during the 6-month healing period. Additionally, the increase in the woven bone to lamellar bone (LB/WB) ratio and the new bone to residual graft material (NB/GM) ratio observed in this 6-month study also provided evidence of increasing bony remodeling and maturity as well as the continuous resorption of the grafting materials. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e6-e11)**

There have been remarkable developments in bone graft techniques and bone substitute materials that are used to fill bony defects adjacent to dental implants. Demineralized bone matrix (DBM), fabricated by removing minerals from bone, is a type of bone allograft that is composed of organic materials, including collagen, bone morphogenic protein, and other growth factors.[1-7] The presence of bone morphogenetic proteins (BMPs) in demineralized allograft has been reported to induce osteoblast and chondroblast differentiation from undifferentiated mesenchymal cells.[1-3] Many studies have provided evidence of bone formation when osteogenic proteins were released from bone marrow during the bone demineralizing process. The significantly low immune rejection has also been attributed to the popular use of DBM as allografts.

Recent findings have also reported weakening of the DBM as a result of the discontinuous release of BMPs. The inconsistency in BMP release from the grafted DBM throughout the healing period suggests a limitation to its osteoinductive properties.[8] Nonetheless, over the past 10 years, the use of DBM in conjunction with a carrier has become a more popular bone-grafting treatment. For instance, there is great use of an inert carrier for the purpose of handling and containing graft, although its use does not provide additional osteoinductivity. The components of this carrier consist of gelatin, glycerol, and poloxamer 407. Commercially available DBM included Regenaform, Regenafil, Grafton, DynaGraft II putty GenSci OrthoBiologics Inc., Goodyear, Irvine, CA, USA, and DynaGraft II gels (GenSci OrthoBiologics Inc.).[9]

Orthoblast II (Isotis Orthobiologics, Irvine, CA, USA) is a DBM product that is composed of 19.5% demineralized bone and 12.5% cancellous allograft. Manufacturers of Orthoblast II (Isotis Orthobiologics, Irvine, CA, USA) claimed that the material is capable of both osteoinductivity and osteoconductivity. In ad-

[a]Associate Professor, Department of Oral and Maxillofacial Surgery, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam City, Republic of Korea.
[b]Professor,Department of Oral and Maxillofacial Surgery, School of Dentistry, Chosun University, GwangJu, Republic of Korea.
[c]Professor, Department of Pathology, School of Medicine, Chosun University, GwangJu, Republic of Korea.
[d]Assistant Professor, Department of Periodontology, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam City, Republic of Korea.
[e]Assistant Professor, Department of Oral and Maxillofacial Surgery, Section of Dentistry, Seoul National University Bundang Hospital, Seongnam City, Republic of Korea.
Received for publication Nov 21, 2009; returned for revision Dec 30, 2009; accepted for publication Jan 23, 2010.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2010.01.012

dition, Orthoblast II uses a reverse-phase medium that has characteristics favored by clinicians. For instance, Orthoblast II becomes soft at room temperature and hardens at body temperature. Changing solidity makes it possible for clinicians to easily manipulate the material and ensure that its structures are stably maintained in the oral environment. Additionally, there is no need to intentionally melt the graft before use. Moreover, when Orthoblast II is applied to the graft area, its viscosity increases and this viscosity is not altered in the presence of bleeding or saline irrigation.[8,10-12] Current clinical research using Orthoblast II is limited. In particular, there are a few published studies using Orthoblast II in conjunction with guided bone regeneration (GBR) in human subjects. In addition, no histomorphometric study has been performed to investigate the stages of healing with time.

Resorbable membranes are manufactured using collagen, lactide/glycolide, polylactic acid, and human dura mater. Depending on the type of material, the resorption period ranges between 8 weeks and 12 months.[13] In recent years, a cross-linked collagen membrane with enhanced strength and a gradual absorption process that maintains barrier function for 4 to 6 months has been developed and has become available commercially. Ossix membrane is a cross-linked collagen membrane that has a higher degree of biocompatibility. It promotes the differentiation and proliferation of fibroblasts and has the ability to increase the thickness of the gingiva. In addition to its hemostatic effect, it has been reported to promote the stabilization of early-stage wounds. Because it is absorbed slowly, the bone regeneration effects are similar to those with nonabsorbable membrane. Despite the exposure of the membrane, there are no great effects.[14-16]

There are differences in the superficial and deep areas of the implant site with respect to the healing process after GBR treatments. In particular, the superficial area of the implant site has frequent marginal bone resorption early within the first year following implant loading. Furthermore, excessive implant loading has been known to accelerate bone loss when the grafted bone is not fully matured. As such, there is a need to evaluate the superficial and deep areas of the implant site during the healing process. In this study, the repair of peri-implant defects using a DBM and a resorbable membrane was investigated. Superficial bone formation beneath the membrane was evaluated at 4 and/or 6 months postoperatively following GBR around the implants using Orthoblast II and resorbable membrane (Ossix; ColBar R & D Ltd., Ramat Hasharon, Israel). Histological evaluations of the bone specimens were performed by measuring the percentage of newly formed bone fractions, the lamellar bone/woven bone ratios, and newly formed bone/graft material ratios.

MATERIALS AND METHODS

Fourteen patients, with a total of 14 specimens, took part in this study from January 2006 to May 2006. All participants signed an informed consent form that was approved by the Institutional Review Board at Seoul National University Bundang Hospital (IRB No. B-0505/016-001). The age of the patients ranged from 31 to 74 years, and 7 of the 14 patients were male.

Implants with GBR (using Orthoblast II and Ossix membrane) were placed in these patients. The specimens were collected from patients who agreed to have a second operation performed at 4 and/or 6 months after the first surgery. This study was completed after 7 specimens from each group were collected.

In all cases, the patients required a bone graft for the regeneration of dehiscence defects around the implants. The dehiscence defects included buccal, lingual, mesial, and distal areas. Photographs were taken before and after bone grafting. The size of the dehiscence defect was not measured (Table I). Orthoblast II was used for grafting. Additionally, Ossix membranes were used for coverage of the bone graft. Membranes were inserted downward into the buccal and lingual flaps. Other materials for fixing were not used in this study. Based on the recorded operation records, specimens were taken from the site in which the Orthoblast II was transplanted. Primary wound closure was carried out with 4-0 absorbable suture (Vicryl, Ethicon, Johnson & Johnson, New Brunswick, NJ, USA).

Four and/or 6 months were allowed for the bone graft to heal before the placement of the implants or exposure of implants in this study. Following the healing period, a second surgical operation was performed by an oral and maxillofacial surgeon at the Seoul National University Bundang Hospital. To collect accurate samples at the site where the grafting was performed using OrthoBlast II, we referred to the surgical records and photographs taken before and after bone grafting. At the site where the bone graft was performed for the secondary surgery, a wedge incisional biopsy was performed. A biopsy specimen was taken from beneath the Ossix membrane during the second operation by performing a wedge incisional biopsy of 3 mm deep using a No. 15 surgical blade. The biopsy specimens were then fixed in 10% buffered formalin and dehydrated in alcohol. The specimens were then demineralized for 12 hours using Calci-Clear Rapid (National Diagnostics, Atlanta, GA), washed in running water, and embedded in a paraffin wax block. Representative areas containing the grafted Orthoblast II were sectioned to 4- to 5-μm thick using an automatic tissue processor (Hy-

Table I. Summary of the cases

Case	Age, y	Gender	Site	Volume of Orthoblast II, mL	Time span between graft and biopsy, mo	Types of implants*
1	51	F	#46-47	1.0	4	3-I Certain
2	74	M	#23	0.5	6	Osstem GS II
3	35	M	#45-46	1.0	4	Implantium
4	59	F	#47	0.5	4	Implantium
5	49	F	#16-17	1.0	6	Osstem US III
6	62	M	#17	0.5	6	Osstem US II
7	51	M	#26	0.5	6	Osstem GS II
8	31	F	#47	0.5	4	Osstem GS II
9	59	F	#14-15	1.0	4	TiUnite
10	45	M	#27-28	1.0	6	Osstem GS II
11	69	M	#36	0.5	6	Osstem GS II
12	44	M	#46	0.5	4	Osstem GS II
13	49	F	#23-24	1.0	4	Osstem US III
14	20	F	#46-47	0.5	6	Osstem GS II

F, female; *M,* male.
*3-I Certain (3i/Implant Innovation, Palm Beach Gardens, FL); Osstem GS II (Osstem Implant system, Osstem, Seoul, Korea); Osstem US II, Osstem US III (Osstem Implant system, Osstem, Seoul, Korea); Implantium (Dentium, Seoul, Korea); TiUnite (Nobel biocare, Gothenburg, Sweden).

percentre XP, Shandon, Cheshire, UK), followed by hematoxylin-eosin and Goldner's trichrome staining before examining under a light microscope (Sigma, St. Louis, MO, USA).

A histomorphometric evaluation, performed by one pathologist, included the use of the computer-assisted Visus Image Analysis System (Image & Microscope Technology, Daejon, Korea). The specimens were photographed by a MagnaFire digital camera system (Optronics, Goleta, CA) and analyzed using a Visus Image Analysis System (Image & Microscope Technology). Density of new bone formation, the ratio of woven bone to lamellar bone (LB/WB), and the ratio of new bone to residual graft material (NB/GM) were measured. The measured LB/WB ratio indicated the occurrence of bony remodeling and maturity and differences between woven and lamellar bone were observed under polarizing microscope. The NB/GM ratio indicated resorption and the osteoconductivity of the bone-grafting materials, and differences between new bone and grafting material were discerned from the different stains. The percentage of newly formed bone fraction, and LB/WB ratio between the 4-month specimens and the 6-month specimens were statistically analyzed using the Mann-Whitney *U* test (nonparametric analysis) ($P < .05$).

RESULTS

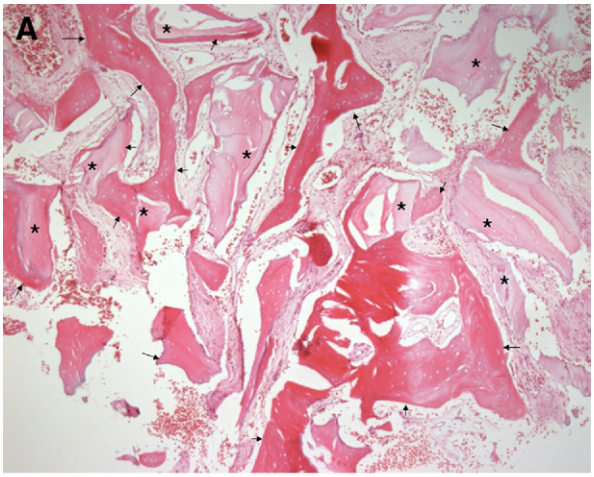
Clinical observations

When flaps were reflected on the grafted sites, Ossix membranes were present in all patients. Beneath the membrane, 1 mm of fibrous tissue was observed and the removal of the fibrous tissue using a No. 15 blade indicated the presence of new weak bone formation with well-developed vascularization.

Histomorphometric evaluations

The histological findings at the 4- and/or 6-month stages are shown in Figs. 1 and 2. New bone formation was continuously observed from specimens 4 months after grafting. The stability of new bone formation, as indicated by the consistency and thickness of the new bone, the formation of bony trabeculae, and lamella bone formation, was observed to be variable at 4 months after grafting, but showed favorable bone formation by 6 months after grafting. The growth of lamella bone (mostly woven bone) was observed as lamella bone mixed with new bone and was continuously thickened. It became more mature with time and with the formation of bony trabeculae. Although massive resorption was found in large areas of specimens during this study period, the resorption was incomplete.

A summary of histomorphometric analysis is shown in Table II. The range of newly formed bone fraction after 4 months was between 12.3% and 67.0% with a mean percentage of 45.0%. At 6 months after surgery, the range of newly formed bone fraction was observed to be between 33.3% and 78.7%, with a mean percentage of 61.7%. Statistical analysis indicated no significant difference ($P = .097$) in the newly formed bone fraction between the 2 postoperative periods. The mean LB/WB ratio 4 months and 6 months after surgery was 0.14 and 0.65, respectively, with significant difference in the LB/WB ratios observed between the 2 postoperative periods ($P = .002$). Additionally, the mean NB/GM ratio



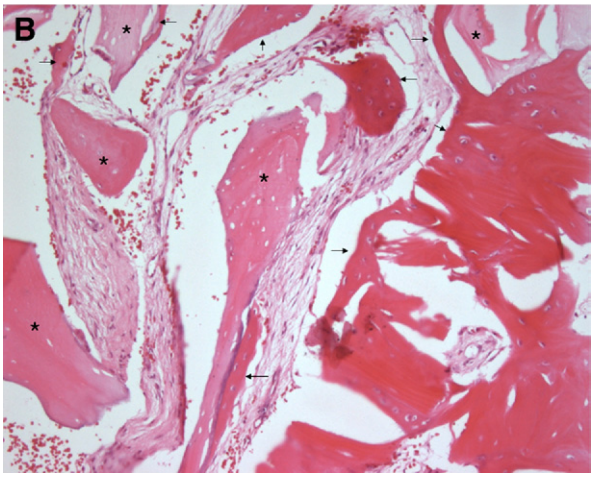
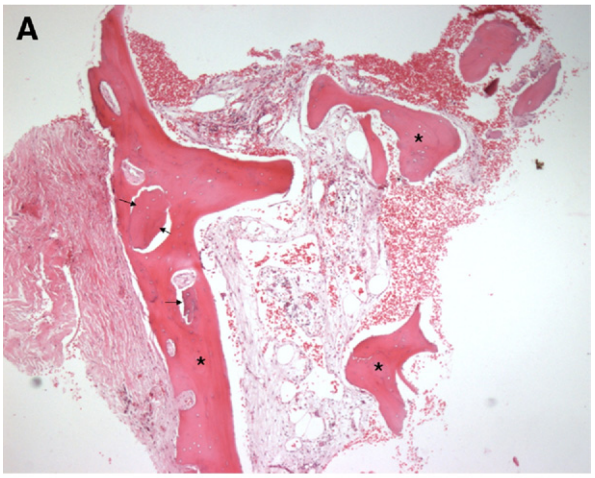


Fig. 1. Histopathologic findings 4 months after GBR (hematoxylin-eosin stain). **A,** Newly formed woven bone (*arrows*) is identified around the implant materials (*asterisks*) (×40). **B,** Magnification of Fig. 1, *A*. Woven bone (*arrows*) is identified around the implant materials (*asterisks*) (×100).



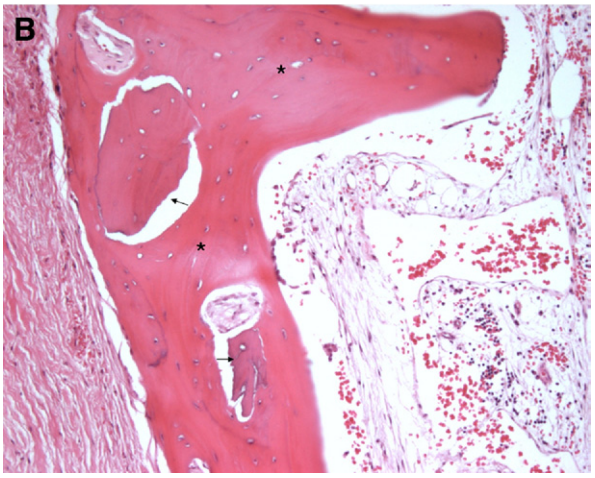


Fig. 2. Histopathologic findings 6 months after GBR (hematoxylin-eosin stain). **A,** Woven bone (*asterisks*) around the implant chip (*arrows*) is identified (×40). **B,** Magnification of Fig. 2, *A*. Woven bone formation (*asterisks*) around the implant chip (*arrows*) is noted (×100).

after the 4-month and 6-month surgeries was 1.24 and 5.52, respectively, with significant difference in the NB/GM ratios observed between the 2 postoperative periods ($P = .001$).

DISCUSSION

Allografts are efficacious when they possess properties that are osteoconductive and osteoinductive. However, it has been reported that there is a lack of rigorous clinical study on the performance of allografts from different manufacturers.[17] The accurate measurement and confirmation of osteoinductivity are essential factors for predicting the success of DBM because of differences in bone formation characteristics between individual donors and tissue banks.[18] Researchers have suggested the combination of DBM with other materials such as tetracycline and osteogenin to enhance growth of new bone.[19-21] Additionally, the use of DBM without carriers in sinus elevation or alveolar ridge reconstruction applications has been reported to be unfavorable, as DBM alone lacks physical strength.[22,23]

Recently, allografts with a high proportion of DBM have been placed on the market. Although these allografts are reported to be favorable, little research has been published.[2,24] Orthoblast II, a commercially available product, integrates DBM with a reverse thermal poloxamer carrier. Poloxamer dissolves into a fluid liquid in water at a low ambient temperature, but becomes a viscous liquid at body temperature. This property facilitates the retention and slow release of DBM and growth factors at surgical sites, suggesting en-

Table II. Summary of the histomorphometric study

	4-month specimens			*6-month specimens*		
	NB fraction	*LB/WB ratio*	*NB/GM ratio*	*NB fraction*	*LB/WB ratio*	*NB/GM ratio*
	27.3	0.05	0.72	57.3	1.08	4.56
	57.0	0.54	1.33	33.3	0.75	4.88
	53.3	0.10	0.96	74.0	0.89	1.56
	12.3	0.01	2.45	61.7	0.37	10.11
	55.7	0.12	0.72	78.7	0.59	3.76
	67.0	0.03	1.08	77.3	0.64	8.09
	42.3	0.10	1.44	49.3	0.23	5.67
Mean	45.0	0.14	1.24	61.6	0.65	5.52
SD	19.2	0.18	0.60	16.6	0.29	2.82

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

hanced osteoinduction.[21] Additionally, a comparative efficacy study of 2 different DBM allografts for treatments of long-bone nonunions in patients who are heavy tobacco smokers indicated that the unique thermal properties of the Orthoblast II as a reverse poloxamer enhanced DBM osteoinduction.[12]

In an animal experiment using Orthoblast II, the DBM group was reported to have more bone density when compared with the autograft group 3 to 6 weeks after surgery.[25] However, at 8 weeks after surgery, the trend was reversed with the autograft group having a higher bone density (54.3%) when compared with the DBM group (45.1%).[25] This observation suggests that the use of DBM favors an increase in new bone density during the early healing period. This increase in new bone density in the presence of DBM was confirmed by other investigators.[26-28] In this study, significant differences in the mean LB/WB ratio and the mean NB/GM ratio were observed after the 4-month and 6-month surgeries. Furthermore, histomorphometric findings suggested the progression of bony maturity and normal bone healing. The significant increase in NB/GM ratio after the 6-month surgery demonstrated the increased accumulation of newly formed bone and resorption of the graft materials during healing.

Recently, Ossix membrane, consisting of bovine type I collagen, was commercially introduced (ColBar R & D Ltd.). Ossix membranes have several strengths. For example, it is more resistant to collagenase digestion at the time of early exposure and thus has extended resorption time. These strengths come from its material makeup that consists of nontoxic metabolites with cross-linking. The manufacturers claimed that such characteristics of the Ossix membrane can remain for 6 months without exposure.[14,15] In a recent experiment to compare the coverings of the dehiscence around the implants using Ossix membranes, BioGide membranes, and expanded polytetrafluoroethylene (e-PTFE) membranes, the average reductions in the defect areas were reported to be 91.50% ± 10.86%, 71.50% ± 8.61%, and 73.70% ± 13.97% for Ossix (OraPharma Inc., Warminster, PA, USA), Bio-Gide (Osteohealth Co., Shirley, NY, USA, and Goretex (W.L. Gore & Associates Inc., Flagstaff, AZ, USA), respectively.[29] Additionally, the average reductions in defect heights reported were 76.4% ± 18.28%, 53.4% ± 9.86%, and 49.4% ± 11.05% for Ossix, Bio-Gide, and Goretex, respectively, suggesting that the Ossix membranes had less bone loss even with an early membrane exposure when compared with other membranes tested. Other studies also indicated a 100% gingival repair with Ossix membrane, whereas an early exposure of e-PTFE did not repair the damaged gingiva.[14]

In this study, Ossix membranes were almost intact, with no resorption observed at 4 and 6 months after surgery. Additionally, bone formation underneath the membrane was observed during these 2 periods. It was also observed that with the increase in healing period, the amount of lamella bone was increased and new bone was mixed with previous bone substances. No signs of chronic granulomatous inflammation around the residual graft material were observed during this study period. Additionally, the increases in new bone formation, LB/WB ratio, and NB/GM ratio not only suggested the occurrence of osteogenesis after 4 months of healing, but also indicated that favorable bone formation accompanied the gradual increase in bone maturity and the resorption of the bone-grafting materials after 6 months of healing. As such, our observations suggested that favorable bone formation occurred with GBR using OrthoBlast II and Ossix membranes. This study included no control group, such as nontreated defects. Accordingly, any comparisons were limited. Moreover, it is also difficult to determine whether the favorable osteogenic effects originated from the OrthoBlast II, the collagen membrane, or a combined effect of the 2 materials.

CONCLUSION

It was concluded from this study that the use of GBR consisting of Orthoblast II and Ossix membranes obtained favorable bone formation during the 6-month healing period. Additionally, the increase in LB/WB and NB/GM ratios observed in this 6-month study also provided evidence of increasing bony remodeling and maturity as well as the continuous resorption of the grafting materials.

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Reprint requests:

Su-Gwan Kim, DDS, PhD
Department of Oral and Maxillofacial Surgery, School of Dentistry
Chosun University
375, SeoSukDong, DongGu
GwangJu City, South Korea 501-759
sgckim@chosun.ac.kr