

## Original

# Augmentation of Peri-implant Bone Defects with Different Bone Grafts and Guided Bone Regeneration: A Pilot Experimental Study in the Dog

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( Accepted for publication, September 20, 2006 )

**Abstract:** Objective; The present investigation was designed to evaluate the regenerating effects of chip bone grafts harvested from implant drilling and biomaterials with or without combined application of guided bone regeneration (GBR) for the augmentation of peri-implant bone defects.

**Materials and methods;** Four mongrel dogs were used. After the mandibular premolars were extracted for 12 weeks, surgical implantation of 4 titanium implants was performed on each side of the mandible. Chip bone was collected during the drilling procedure. Three-wall bone defects, about 2 mm high, 2 mm wide, and 3 mm long, were created at the buccal side of each implant. The osteotomy sites were separately filled with autogenous chip bone, Bio-oss mineral bone or mixture of both except one defect was left empty. The operation areas of the left mandibula were covered with titanium membranes. The right mandibles without membrane were used as controls. The dogs were sacrificed at either 9 or 16 weeks after the second operation. After the specimens containing experimental implants were resected, the resorption amount of the bone graft was measured. Then the specimens were processed for ground sectioning. The sections were stained with methylene blue and alkaline fuchsin. Histological examination was performed and photographs were taken under photomicroscope.

**Results;** The mean vertical distances of each bone defects were 2.64±0.17 mm in the D1 (control) group, 1.81±0.21 mm in D2 (autograft) group, 1.72±0.34 mm in D3 (Bio-oss) group, 1.51±0.22 mm in D4 (mixture) group. Statistical analysis demonstrated that there was significant difference between the results of D1 and the results of other three groups (p<0.01). No statistical differences were revealed between autograft group and Bio-oss group, as well as Bio-oss group and mixture group. It is confirmed there was significant difference between autograft group and mixture group (p<0.05). Histological observations revealed both quantity and quality of regenerated bone in the mixture grafted specimens were superior to that of single Bio-oss grafted or single autologous bone grafted specimens. Whether the membranes were utilized or not, vertical distances of the defects were larger at 16 weeks than those at 9 weeks in all the groups. Bone grafts with titanium membranes were subjected to greater resorption than those without membrane coverings at either 9 weeks or 16 weeks. Statistical analysis indicated there were significant differences in the results between the two groups (p<0.01). **Conclusions:** This investigation demonstrated the utilization of bone chip collected during implant drilling in combination with biomaterial was a feasible method for repairing the limited bone defect around the implant. Using the mixture of autogenous bone and Bio-oss mineral as grafting material may significantly promote the bone regenerating effects. The functions of titanium membrane need to be further investigated.

**Key words:** Bone graft, Dental implant, Guided bone regeneration.

## Introduction

A successful outcome of oral implant therapy requires sufficient bone to cover implant and support a fixed prosthesis. Autogenous bone grafts as well as other bone substitutes combined with membrane technique have been extensively used to augment a

compromised alveolar ridge and to prepare the site for dental implant installation<sup>1-3)</sup>. Autologous bone can be harvested from iliac crest, mental portion of mandibula and ribs. Although the high rate of osseointegration between autogenous grafts and implant is favorable, the deficiencies of these kinds of technique still exist, such as exploration of a second operation field and additional injury for the patients.

For the small defects left around the dental implant, it is often

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suggested that bone graft be obtained from adjacent area of the implant. Autologous bone collected during the drilling procedure may be the favorite graft sources since there is no additional injury added on the patients. But autogenous graft of this type could not be expected to provide a large amount, on the other hand, it has been reported that autogenous grafts made up of cortical bone and cancellous bone would undergo marked resorption during healing<sup>4,5</sup>, and therefore other bone graft substitutes were also considered. Bio-oss is a bovine-derived bone substitute that has been used to augment the resorbed alveolar ridge in man and experimental animals<sup>6-8</sup>). Clinical and experimental studies have indicated that Bio-oss may be incorporated in the bone tissue and that intimate contact will be established between the biomaterial newly formed lamellar bone. But it remains unclear among the three patterns of graft: autograft, Bio-oss mineral and mixture of the former two, which one is the best in accelerating bone regeneration.

The present animal experiment was designed to evaluate the regenerating effects of chip bone grafts harvested from implant drilling and biomaterials of Bio-oss mineral with or without combined application of guided bone regeneration (GBR) for the augmentation of peri-implant bone defects.

#### Materials and methods

Four young, healthy, mongrel dogs were selected for this study. During the surgical procedures, the animals were anesthetized with intra-abdominal injection of amobarbital sodium at a dosage of 30 mg/kg and anesthesia with 2% xylocaine was used at the same time.

The study was performed in two surgical stage. In the first stage, bilateral mandibular premolars were sectioned and extracted. Postsurgically, the dogs were placed on a soft diet for the remainder of the study. After the newly edentulated ridges had healed for 12 weeks, radiograph were taken to ensure that no tooth root had been inadvertently left.

In the second stage, surgical implantation of cylinder titanium implants (2.0 mm diameter and 7.0 mm insertion depth, Zhongbang Biomaterial Inc. Xian, China) was performed on the edentulous alveolar ridges. After a crestal incision, full-thickness mucoperiosteal flaps were elevated and 4 implants were placed 5 mm apart in each edentulous ridges. Chip bone was collected

during the drilling procedure and harvested by means of a filter attached to a dedicated suction line. Then, low-speed rotary and hand instrument were used to create a three-wall bone defect at the buccal side of each implant. Copious sterile saline irrigation was provided during the preparation of the bone defect. The dimensions of the bone defects were 2 mm apicocoronal, 2 mm mesiodistal, and 3 mm buccolingual. These dimensions were verified by careful clinical measurement with vernier calibers.

From oral-caudal direction, the osteotomy sites were named D1, D2, D3 and D4 in turn. D1 was used as control and no bone graft was placed in. D2, D3 and D4 were separately filled with autogenous chip bone, Bio-oss mineral bone (Geistlich AG, Wollhusen, Switzerland), and mixture of autogenous bone and Bio-oss mineral bone (volume proportion 1:1). Being moistened by saline liquid, the grafts were densely packed to maintain the close contact with the defect walls. Subsequently, the operation areas of the left mandibula were covered with titanium membranes (Zhongbang Biomaterial Inc. Xian, China). The right mandibles without membrane were used as controls (Table 1). The membranes were cut and shaped to fit the contour of the implanting areas and then secured by the membrane fixing pins that were placed in the prepared sites 2-3 mm from the defect margins. The mucoperiosteal flaps were repositioned and sutured. After the operation, each animal was given an intramuscular injection of penicillin G (800,000 units) twice per day.

Two dogs were sacrificed at either 9 or 16 weeks after the second surgery. The animal's jaws were removed and specimens containing experimental implants were resected for observation. The distance of 'coronal' level of the graft to the upper surface of the implant was measured by vernier calibers to represent the amount of the bone graft resorption. Afterwards, the specimens were fixed in 10% neutral buffered formalin for a week, and then dehydrated in increasing grade of ethanol and finally embedded in methylmethacrylate (Fisher scientific, Fair Lawn, NJ, USA). The specimen blocks were sectioned parallel with the long axis of implant in a buccolingual direction. From each grafted site, three sections representing the central area were obtained. Undecalcified sections of 150  $\mu$ m thickness were cut by a low speed diamond blade saw (Leitz 1600, Leica, Germany). Sections were grinded to 50 $\mu$ m using a roll grinder containing sandpapers of decreasing grit sizes. The cut surface was mounted on a slide,

Table 1 Experimental design: number of defects in each group

		D1 (control)	D2 (autograft)	D3 (Bio-oss)	D4 (mixture)
9w	with membrane	2	2	2	2
	without membrane	2	2	2	2
16w	with membrane	2	2	2	2
	without membrane	2	2	2	2

Table 2 Vertical distances of bone defects with membrane at 9 weeks (mm)

	D1 (control)	D2 (autograft)	D3 (Bio-oss)	D4 (mixture)
dog 1	2.60	1.82	1.54	1.38
dog 2	2.48	1.76	1.50	1.32
mean	2.54 $\pm$ 0.06	1.79 $\pm$ 0.03	1.52 $\pm$ 0.02	1.35 $\pm$ 0.03

Table 3 Vertical distances of bone defects without membrane at 9 weeks ( mm )

	D1 (control)	D2 (autograft)	D3 (Bio-oss)	D4 (mixture)
dog 1	2.54	1.54	1.38	1.26
dog 2	2.26	1.48	1.34	1.20
mean	2.40±0.14	1.51±0.03	1.36±0.02	1.23±0.03

Table 5 Vertical distances of bone defects without membrane at 16 weeks ( mm )

	D1 (control)	D2 (autograft)	D3 (Bio-oss)	D4 (mixture)
dog 3	2.64	1.66	1.78	1.54
dog 4	2.78	1.74	1.72	1.62
mean	2.71±0.07	1.70±0.04	1.75±0.03	1.58±0.04

polished and stained with methylene blue and alkalescence fachsinn. Histological examination was performed and photographs were taken under photomicroscope. (Olympus, BH-2, Japan).

**Results**

**Clinical observation**

At 9 weeks, no titanium membrane was exposed and mucosa of the operation sites exhibited normal shape and colors. But the membranes of the 16 weeks group were found to be exposed and not properly adapted over the bone graft at 10 and 12 weeks. After the exposed membranes were removed, the wounds were sutured and no infections were present. At the time of sacrifice, all the implants appeared to be steady and undergoing normal healing. None of the 40 implants lost. When the titanium membranes were dismantled, a layer of ‘pseudo-periosteum’ was found to cover the grafting sites which was verified to be connective tissue later.

**Clinical measurement**

The vertical distances from the ‘coronal’ level of the graft (or the apical bottom of the defect) to the upper surface of implant were measured to represent the resorption volume of the bone or bone grafts. The results of clinical measurement are reported in table2-5.

From the tables, outcomes were obtained as follows.

1. Bone resorptions occurred in all the defects without bone grafts. In the D1 group at 9 weeks, the vertical distances of bone defect were 2.54±0.06 mm with membrane covering, and 2.40±0.14 mm without membrane covering. The vertical distances of bone defect with and without membrane covering were 2.90±0.02 mm and 2.71±0.07 mm at 16 weeks.
2. Without considering the factors of time and membrane, the

Table 4 Vertical distances of bone defects with membrane at 16 weeks ( mm )

	D1 (control)	D2 (autograft)	D3 (Bio-oss)	D4 (mixture)
dog 3	2.88	2.26	2.20	1.78
dog 4	2.92	2.24	2.30	1.98
mean	2.90±0.02	2.25±0.05	2.25±0.05	1.88±0.10

vertical distances of each experimental site were 2.64±0.17 mm in the D1 (control) group, 1.81±0.21 mm in D2 (autograft) group, 1.72±0.34 mm in D3 (Bio-oss) group, 1.51±0.22 mm in D4 (mixture) group. Statistical analysis demonstrated that there was significant difference between the results of D1 and the results of other three groups (p<0.01). This means that grafting procedure for the bone defect around implant may significantly maintain or increase the bone volume. Although no statistical differences were revealed between autograft group and Bio-oss group, as well as Bio-oss group and mixture group, it is confirmed that was significant difference between autograft group and mixture group (p<0.05). This result indicates the superior effect of using mixture of autograft and Bio-oss mineral for bone regeneration (Figure 1, 2).

3. Whether the membranes were utilized or not, vertical distances of the defects were larger at 16 weeks than those at 9 weeks in all the groups. This means the bone resorption was continuing along with time after grafting operation.

4. Bone grafts with titanium membranes were subjected to greater resorption than those without membrane coverings at either 9 weeks or 16 weeks. Statistical analysis indicated there were significant differences in the results between the two groups

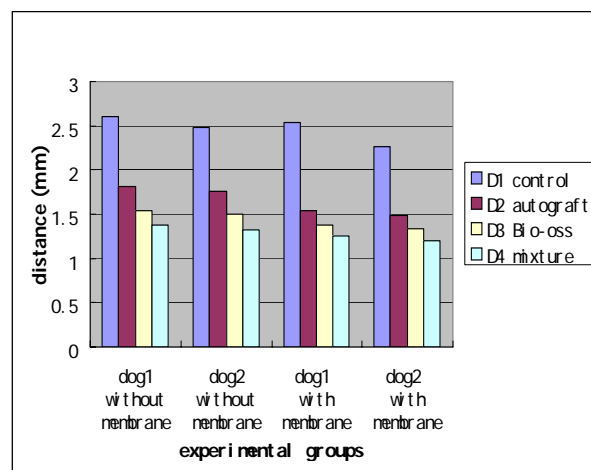


Fig. 1. Bone resorptions occurred in all the defects without bone grafts. The vertical distances of bone defect were 2.54±0.06 mm with membrane covering, and 2.40±0.14 mm without membrane covering at 9 weeks. Bone resorption was reduced obviously when mixture of autograft and Bio-oss mineral was used for grafting.

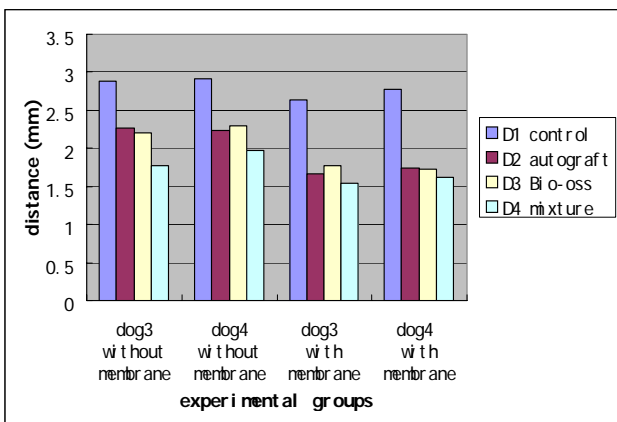


Fig. 2. At 16 weeks vertical distances of bone defects increased compared with those at 9 weeks. The resorption distances of mixture groups remained the lesser than those of the other two grafts.

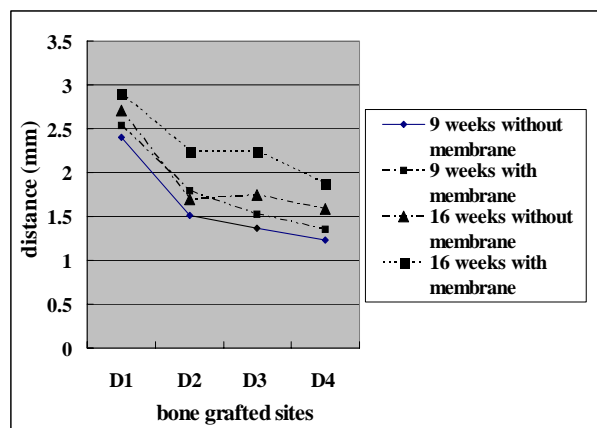


Fig.3. Bone grafts covered with titanium membranes were subjected to greater resorption than those without membrane coverings at either 9 weeks or 16 weeks.

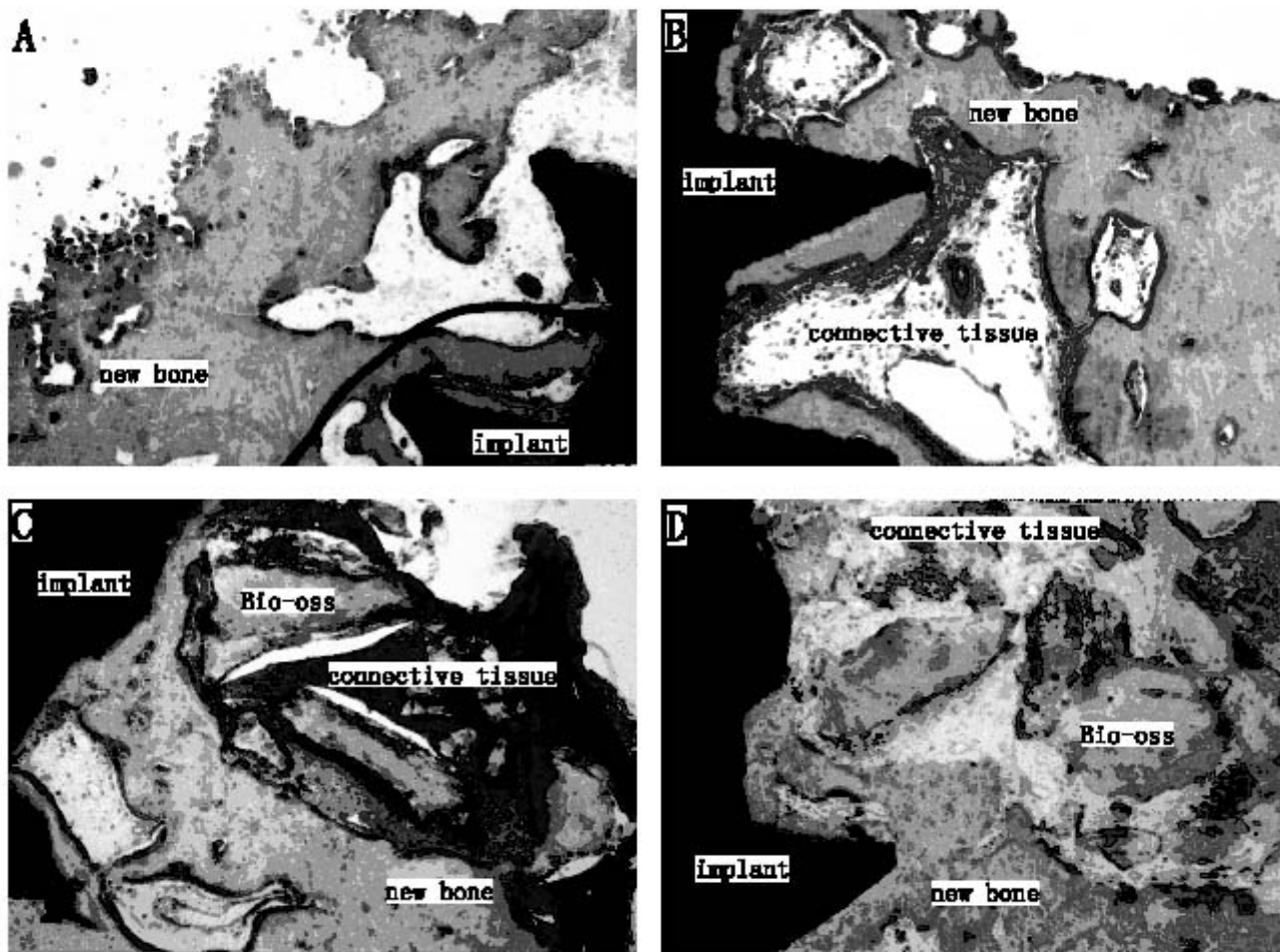


Fig. 4. Histological micrographic images of control group (A), autograft group (B), Bio-oss group (C) and mixture group (D) at 9 weeks. Minimal bone formation was noted in the defects without any graft. The newly formed bone composed of irregular thin trabecula could be seen in the defects repaired by autograft. In the Bio-oss group newly formed bone was deposited on the surface of the Bio-oss particles and loose fibrous connective tissue were present in the space. In the mixture group active bone formation appeared to be present on the surface of Bio-oss particles, new bone connected to form bone bridges. The slices were stained with methylene blue and alkaescence fuchsin with magnifying rate of 4 times.

( $p < 0.01$ ).

**Histological observations**

Osseointegration appeared to be present in all 40 specimens,

and no connective tissue or inflammatory cells was disclosed in the vicinity of the interface between implants and host bone. A layer of fibrous connective tissue with few cells was present on the surface of the grafts which were covered with titanium

membranes, whereas on the controlateral sides without membranes covering, direct contact was showed between graft and periosteum.

#### 1. D1 (control group)

After 9 weeks of healing, minimal bone formation was noted in the defects without any graft. A thin layer of branching slender trabecula with small amount of osteoblastic and osteoclastic cells in the marrow could be seen on the surface of host bone demonstrating the bone remodeling process (Figure 4A). At 16 weeks, thick trabecula with round osteoblasts on its surface interweaved with bone marrow. Osteocytes were present in the network of new bone.

#### 2. D2 (autograft group)

The newly formed bone composed of irregular thin trabecula could be seen in the defects repaired by autograft at 9 weeks. In the basal area, the regenerated bone with flat osteoblasts on its surface showed on a net-work like structure. The peripheral area of the graft exhibited loose connective tissue and bone with the presence of osteoclastic lacunae (Figure 4B). In the autograft sections at 16 weeks, a large-meshed network of new bone with thick trabecula was observed. Harvard ducts and osteocytes were seen scattering in the network.

#### 3. D3 (Bio-oss group)

Blocks of Bio-oss particles with brown color were observed in the specimens with single Bio-oss graft. At 9 weeks, the irregular margin of the particles presented a scalloped appearance due to the presence of resorption lacunae. In the basal area, newly formed bone was deposited on the surface of the Bio-oss particles. Loose fibrous connective tissue and capillaries were present in the space. In the outer regions, only blue connective tissue was observed in the space and no new bone was present (Figure 4C). At 16 weeks, further resorptions of Bio-oss particles were found, even in some regions, complete resorption occurred. A varying amount of newly formed bone had established contact between biomaterial and implant surface. A larger amount of newly formed bone occupied the intertrabecular areas of the bovine bone. Some of the new bone appeared to be mineralized and matured with numbers of osteocytes present in the bone lacunae.

#### 4. D4 (mixture group)

The grafted bone and biomaterial particles were observed in the mixture grafting specimens at 9 weeks. Active bone formation appeared to be present in the spaces of Bio-oss particles in some regions, new bone connected to form bone bridges (Figure 4D). At 16 weeks, the mixture grafted specimens showed similar under-microscope exhibition to the specimens with single Bio-oss graft. The difference was that both quantity and quality of regenerated bone in the mixture grafted specimens were superior to that of single Bio-oss grafted specimens. Most regions of graft space were filled with mineralized mature bone with limited Bio-oss remainders scattered in. The numbers of osteocytes were more than that of Bio-oss group.

#### 5. Membrane (used or not)

The volumes of newly formed bone in the specimens covered by titanium membrane appeared to be less than that in the specimens without membrane. In the specimens covered with membranes, no bone bridge was observed between the Bio-oss particles which margins subject to less resorption at 9 weeks. At 16 weeks, larger volumes of mineralized mature bone with more haversian canals and osteocytes were observed in the specimens without membranes. Without membrane's protection, the Bio-oss particles suffered a greater resorption, even completely disappeared in some regions.

### **Discussion**

This investigation demonstrated the utilization of bone chip collected during implant drilling in combination with biomaterial was a feasible methods for repairing the limited bone defect around the implant.

The bone inductive and conductive properties of autologous bone graft have been reported to improve the bone regeneration<sup>9</sup>. The autologous bone granules obtained from implant drilling have dimensions of 0.5-1.5 mm, which were composed of cancellous and cortical bone<sup>10</sup>. Though cancellous bone has displayed the advantage of rapid revascularization, manageable molding and strong ability of anti-infection, it has a low mechanical intensity and was easy to be absorbed in the future. Otherwise, the cortical bone has superior mechanical properties, and would endure the load early<sup>11</sup>. In our study, autogenous bone chip were observed to be in connection with the recipient bone and could not be distinguished. This indicated that the grafts had grown together with the host bone. However, the remaining bone in 16 weeks reduced comparing with that in 9 weeks, which revealed the continuous resorption of the bone chips. The finding that marked amounts of resorption occurred in the peripheral area of the bone graft during healing is in accordance with the previous data<sup>12</sup>. Continuous resorption also occurred in the defects without bone grafts, which further illuminated the necessity of bone grafting for the peri-implant defect.

Considering the limited volume of autologous graft and the high self resorption rate, bone substitute, Bio-oss mineral, was applied to accelerate the bone regeneration in the present experiment. There have been contradictory statements on the function of Bio-oss. Some authors believed that Bio-oss, when incorporated in the host bone, will be maintained as an inactive filler material and only becomes utilized and resorbed when the tissue is undergoing extensive remodeling<sup>13</sup>, others proposed that the beneficial effect of deproteinized bovine bone mineral was limited to the initial stage of healing and at later stages the biomaterial may have been an obstacle to bone formation. In the present animal experiment, it was observed that the Bio-oss graft maintained its framework of trabecular bone at early stage, with the tiny scalloped resorptions at the margin of the particles. Further resorptions of Bio-oss particles were found at 16 weeks, even in

some regions, complete resorption occurred. This finding is in agreement with previous results that recorded the slow resorption of this biomaterial<sup>7,13</sup>.

The amount of the newly formed bone in the trabecular network of the Bio-oss block was increasing during the period between 9 weeks and 16 weeks postoperative, though the vertical height of graft reduced according to the clinical measurement. This result suggested that after 16 weeks of grafting there was active new bone forming in the basal area and obvious loss of materials in the peripheral area of the graft. The outcomes from the measurement and histological observation demonstrated that mixture of autologous bone and Bio-oss mineral had the superior capability to induce regenerated bone and maintain the height of bone graft. The reasons for the better regeneration effects of mixture grafts may be explained as follows. Bio-oss particles provided the frame and space for the new bone to access, on the other hand, autograft which contained bone growth factors and bone cells possessed the best bone forming traits<sup>11,14</sup>. Therefore it may be speculated that mixture of autologous bone and Bio-oss mineral would be the ideal graft for peri-implant defects repairing.

The use of barrier membrane is one of the important techniques in guide bone regeneration (GBR). The membrane technique in conjunction with the placement of dental implants has gained approving outcomes in clinical and experimental studies<sup>15-17</sup>. In the present study, both quantity and quality of regenerated bone in specimens without membrane's protection were superior to that of the specimens covered by titanium membrane. This may attribute to improper membrane operating technique.

#### Acknowledgement

The authors thank the Chinese PLA General Hospital for the opportunity to use the Leitz I600 blade saw and providing the histological technique support.

This work was also partially supported by grants in aid for scientific researches from the Ministry of Education, Culture, Sports, Science and Technology (#15209060, 17406027, 17591910, 17591911). This work was also supported by grant-in aid from Chugoku Industrial Innovation Center (#1039908059 and #1039908061).

#### References

1. Hammerle CH, Chiantella GC, Karring T, Lang NP. The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. *Clin Oral Implants Res* 9: 151-162, 1998
2. Hockers T, Abensur D, Valentini P, Legrand R, Hammerle CH. The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study in beagle dogs. *Clin Oral Implants Res* 10: 487-498, 1999
3. Schepers E, Barbier L, Ducheyne P. Implant placement enhanced by bioactive glass particles of narrow size range. *Int J Oral Maxillofac Implants* 13: 655-665, 1998
4. Donos N, Kostopoulos L, Karring T. Augmentation of the rat jaw with autogeneic cortico-cancellous bone grafts and guided tissue regeneration. *Clin Oral Implants Res* 13: 192 - 202, 2002
5. Araújo MG, Sonohara M, Hayacibara R, Cardaropoli G and Lindhe J. Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol* 29 :1122 –1131, 2002
6. Hockers T, Abensur D, Valentini P, Legrand R and Hammerle CH. The combined use of bioresorbable membranes and xenografts or autografts in the treatment of bone defects around implants. A study in beagle dogs. *Clin Oral Implants Res* 10:487-498, 1999
7. Carmagnola D, Berglundh T, Araujo M, Albrektsson T, Lindhe J. Bone healing around implants placed in a jaw defect augmented with Bio-Oss: An experimental study in dogs. *J Clin Periodontol* 27:799-805, 2000
8. Mayfield LJ, Skoglund A, Hising P, Lang NP, Attstrom R. Evaluation following functional loading of titanium fixtures placed in ridges augmented by deproteinized bone mineral. A human case study. *Clin Oral Implants Res* 12:508-514, 2001
9. Von Arx T, Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Lateral ridge augmentation and implant placement: an experimental study evaluating implant osseointegration in different augmentation materials in the canine mandible. *Int J Oral Maxillofac Implants* 16:343-354, 2001
10. Mellonig JT. Human histologic evaluation of a bovine-derived bone xenograft in the treatment of periodontal osseous defects. *Int J Periodontics Restorative* 20:19-29, 2000
11. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. *Int J Oral Maxillofac Implants* 13:11-45, 1998
12. Donos N, Kostopoulos L, Karring T. Augmentation of the mandible with GTR and onlay cortical bone grafting An experimental study in the rat. *Clin Oral Implants Res* 13: 175 -184, 2002
13. Araujo MG, Carmagnola D, Berglundh T, Thilander B and Lindhe J. Orthodontic movement in bone defects augmented with Bio-Oss. An experimental study in dogs. *J Clin Periodontol* 28:73-80, 2001
14. Richardson CR Mellonig JT, Brunsvold MA, McDonnell HT and Cochran DL. Clinical evaluation of Bio-Oss: a bovine-derived xenograft for the treatment of periodontal osseous defects in humans. *J Clin Periodontol* 26:421-428, 1999
15. Kohal RJ, Trejo PM, Wirsching C, Hurzeler MB and Caffesse RG. Comparison of bioabsorbable and bioinert membranes for guided bone regeneration around non-submerged implants. An experimental study in the mongrel dog. *Clin Oral Implants Res* 10:226-237, 1999

16. Von Arx T, Kurt B. Implant placement and simultaneous ridge augmentation using autogenous bone and a micro titanium mesh: a prospective clinical study with 20 implants. Clin Oral Implants Res 10: 24-33, 1999
17. Mardas N, Kostopoulos L, Stavropoulos A and Karring T. Evaluation of a cell-permeable barrier for guided tissue regeneration combined with demineralized bone matrix. Clin Oral Implants Res 14 :812 -818, 2003