Bone Regeneration Using Bilayer Bone Augmentation around Dental Implants: A Preliminary Study by Micro-CT in Dogs

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The purpose of this preliminary study was to compare the effects of the bilayer bone augmentation technique (BBA) for the treatment of dehiscence-type defects around implants and evaluate the role as a membrane of the xenogenic bone positioned as the outer layer in the BBA technique using a micro-computed tomography (micro-CT). Four standardized dehiscence defects were prepared on each mandible bilaterally in 3 dogs and 1 implant was placed per defect, where each defect was treated with autograft (AB), xenograft (XB), BBA technique, or negative control without a membrane. Two months post-regenerative surgery, sectioned bone blocks were obtained. The image acquisitions were then scanned by micro-CT. Bone volume (BV), horizontal bone width (HBW) and vertical bone height (VBH) were measured through the analyses program. The BV were 11.08 mm³, 10.42 mm³, 8.1 mm³, and 7.01 mm³ in XB, BBA, control, and AB group in sequence of high value, respectively. HBW were 1.33 mm, 1.3 mm, 1.06 mm, and 1.03 mm in XB, BBA, AB, and control group, respectively. VBH were 4.88 mm, 4.85 mm, 4.74 mm, and 4.67 mm in XB, BBA, AB, and control group, respectively. However, there was no significant difference between the 4 groups. VBH tended to be higher in sequence of control, AB, BBA, and XB group (*p* for trend <0.05). The results showed the usefulness of the BBA technique involving mechanical support for prolonged space maintenance of xenogenic bone, for the treatment of dehiscence-type defects around implants. However, further studies with a larger sample size are required to confirm the results.

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Key Words: Bilayer bone augmentation; Dehiscence defect; Dental implant; Micro-computed tomography

INTRODUCTION

Recovery of missing teeth is one of the most critical concerns in clinical dentistry. Currently, dental implant is generally applied for replacing missing teeth and the long term results of many studies are described. However, resorbed and atrophic alveolar ridge due to periodontitis and trauma, may create various challenges to the ideal placement of dental implants [1,2]. Several clinical and experimental studies have described dehiscence-type defect as a result of implant placement at a buccolingually resorbed alveolar ridge [3-5]. Dehiscence area without correction causes a negative influence on survival of dental implants by preventing proper osseointegration and

causing esthetic problems [6]. It is inevitably necessary to have an additional bone graft on the dehiscence-type defect area.

For the successful dehiscence-type defect resolution, many clinical and experimental studies have been done using guided bone regeneration (GBR) with various bone substitutes and membranes [7]. The main role of barrier membrane used in the GBR is to occlude the space from connective tissues and maintain stability for blood clots and grafted material, if used together [5,6,8]. However, barrier membrane has some disadvantages, such as expense and difficulty in handling; additional surgery is also required to remove non-absorbable membrane. A recent study showed a high rate of complication related with barrier membranes, including the exposure of the membrane [6].

Autogenous bone has the combined functions of osteogenesis, osteoinduction and osteoconduction. Hence, it is regarded as the “gold standard” in bone regeneration [9,10]. However, it still has some drawbacks such as the formation of a donor site and limited amounts to harvest [9,11]. Xenograft is good for

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osteoconduction and provides stability as a scaffold during a period of bone regeneration. It is slow to exchange host bone and does not have the effects of osteoinduction [12,13]. The purpose of this preliminary study was to compare the effects of the bilayer bone augmentation technique (BBA) for the treatment of dehiscence-type defects around implants and evaluate the role of a membrane of the xenogenic bone positioned at the outer layer in the BBA technique using a micro-computed tomography (micro-CT).

MATERIALS AND METHODS

Animals

The study was approved by the Committee on the Use and Care of Animals and the Institutional Review Board (IRB) of the Catholic University of Korea (2013-0079-01).

Three male beagle dogs, approximately 12 to 15 months old and weighing approximately 9.5 kg, were used in this study. All dogs were checked by a veterinarian for health status before beginning the study. An accommodation time of 2 weeks prior to the surgical procedure was allowed.

Surgical protocol

All surgical procedures, including tooth extraction and subsequent experiments, were performed under general anesthesia. All animals were anesthetized using an intravenous injection of atropine (0.04 mg/kg; Jeil Pharmaceutical, Daegu, Korea) and an intramuscular injection of a combination of xylazine (Rompun®, Bayer Korea, Seoul, Korea) and ketamine (Ketara®, Yuhan, Seoul, Korea). General anesthesia was maintained with inhalation anesthesia (Infran®, Hana Pharmaceutical, Seoul, Korea). The mandibular premolars and first molars were extracted bilaterally from each dog. After 2 months of healing following teeth extraction, the animals were prepared for the experiments. A crestal incision was made and a full-thickness flap was elevated. The edentulous ridges used were flattened using a ridge-contouring bur with a rotary motor, and 8 sites in each dog were identified for the placement of dental implants. Surgical osteotomies for the implant placement were performed according to the manufacturer's instructions. After implant preparation, 4 rectangular bone defects measuring 5 mm in height from the crestal bone, 3 mm in width mesiodistally, and 2 mm in depth from the surface of the buccal bone were surgically created bilaterally in each dog. A dental implant (TSIII®, Osstem, Seoul, Korea) with a diameter of 3.8 mm and a length of 10 mm was placed at each of the 4 bilateral sites. The final tightening of the fixture into bone is performed with a torque wrench and the primary stability was ≥ 35 Ncm. The implants were placed in such a manner that their upper sur-

faces were in line with the alveolar crests (Fig. 1). The 4 sites in each dog unilaterally were allocated to 4 different treatment groups: 1 site received autogenous particulated bone obtained from mandibular premolar region alone (AB); 1 site received xenogenous bone (Inducera®, Oscotech, Seoul, Korea; bovine porous bone mineral coated dually with biocompatible calcium phosphate) alone (XB); 1 site received autogenous particulated bone (inner layer contacted with buccal surface of implant fixture) and xenogeneous bone (outer layer) (the BBA technique); and 1 site left untreated (control) (Fig. 2). The flaps were repositioned and sutured with resorbable suture materials (Vicryl® 4/0, Ethicon, Somerville, NJ, USA). Two months post-regenerative surgery, animals were sacrificed by an injection of KCl (Potassium Chloride 3.0®, Daihan Pharm, Seoul, Korea) after anesthesia and sectioned bone blocks were obtained. Animals were maintained on a soft diet (Prescription Diet Canine i/d®, Hill's Pet Nutrition, Topeka, KS) from the time of the surgical procedure until the end of the study.

Figure 1. Four dental implants were installed at the prepared hole with standardized dehiscence defects.

Figure 2. After installing dental implants, the bone grafting procedure was done at each dehiscence-type defect for bone regeneration (from the left site; xenograft, autograft, control, bilayer bone augmentation).

Micro-computed tomography analysis

Block sections, including the segments with implants, were preserved and fixed in 10% neutral buffered formalin. Image acquisitions were scanned by micro-CT (Skyskan 1076®, skyskan, Kontich, Belgium). The acquisition settings were the following: X-ray source voltage 100 kVp, current 100 μ A, 0.5 mm thick aluminium filter was used for beam hardening reduction. The pixel size was 18 μ m, exposure time was 14.7 seconds, the rotation step 0.5°, with a complete rotation over 360°. Bone volume (BV), horizontal bone width (HBW) and vertical bone height (VBH) at the area of dehiscence-type defect were measured from the acquired images with the analyzing program (CTAn version: 1.13.2.1, Bruker, Kontich, Belgium).

For BV, a fraction that showed similar density of corticocancellous bone in the dehiscence-type defect, was measured through 3D tomography reconstruction program (NRecon version: 1.6.6.0, Bruker, Kontich, Belgium) (Fig. 3). For HBW, the width between thread (which was positioned from the

shoulder part of dental implant by 3 mm below) and the point that showed bone density, was measured buccally. For VBH, the height from the bottom of defect to the point that showed bone density, was measured as high as possible (Figs. 4 and 5).

Statistical analysis

Statistical analysis was performed on these 3 parameters for the experimental and control groups. Firstly, the mean and standard deviation of each group were suggested, using General Linear Model, p value and p for trend derived from the differences and changes of each group were presented. SAS Version 9.3 (SAS Institute, Cary, NC, USA) was used as the statistical analysis program. Significant level of p value and p for trend was set at 0.05.

RESULTS

Among the total 24 bone graft sites from 3 dogs, 5 dental

Figure 3. Three dimensional image of block section, including the segments with implant and graft material. For bone volume, a fraction of corticocancellous bone density in the dehiscence-type defect was measured. A: Bone volume space on sagittal plane view. B: Bone volume space on coronal plane view.

Figure 4. Three sectional images of peri-implant dehiscence defect. HBW was measured by using transverse and axial section. VBH was measured by axial and coronal section. A: Traverse section. B: Axial section. C: Coronal section. HBW: horizontal bone width, VBH: vertical bone height.

implants failed to achieve osseointegration. Finally, the remaining 19 dental implants of control and experimental groups were included in this study. The mean value and standard deviation of each parameter from each group were shown in Table 1. The regenerated BV were $11.08 \pm 3.2 \text{ mm}^3$, $10.42 \pm 3.5 \text{ mm}^3$, $8.1 \pm 5.99 \text{ mm}^3$, and $7.01 \pm 5.61 \text{ mm}^3$ in XB, BBA, control, and AB group in sequence of high value, respectively. HBW were $1.33 \pm 0.25 \text{ mm}$, $1.3 \pm 0.26 \text{ mm}$, $1.06 \pm 0.23 \text{ mm}$, and $1.03 \pm 0.44 \text{ mm}$ in XB, BBA, AB, and control group, respectively. VBH were $4.88 \pm 0.1 \text{ mm}$, $4.85 \pm 0.12 \text{ mm}$, $4.74 \pm 0.17 \text{ mm}$, and $4.67 \pm 0.13 \text{ mm}$ in XB, BBA, AB, and control group, respectively (Fig. 6, Table 1), with no significant difference between the 4 groups (Table 2). No specific trend was observed between BV and HBW through additional trend analysis. VBH tended to be higher in sequence of control, AB, BBA, and XB group (Table 1).

DISCUSSION

Maintenance of space, creating a barrier from connective tissue, and availability of sufficient osteoinduction factors in a bone defect are very important for successful GBR [7,8,14]. In the present model, a method combining each characteristic of these 2 kinds of material was designed and accordingly, autog-

enous bone obtained from the dog as an inner layer (closed to implant surface) and xenograft materials (bovine bone material coated by calcium phosphate) as outer layer were applied in dehiscence-type defects. The purpose of the BBA technique in bone regeneration is to maintain stability in the outer layer using xenograft materials and to maximize osteogenic effects in the inner layer using autogenous bone.

The bovine bone as a xenograft is generally used for graft material of absorbed alveolar ridges and various bone defects after a dental implant placement [15-18]. Good biocompatibility and superior osteoconduction of this bone substitute has shown in several previous studies [17-20]. The resorption rate and remodeling of bone mineral are generally slowly processed and it is easy to find bovine bone completely retained in the bone graft area for a long time without resorption [20,21]. Piatelli et al. [22] reported that newly formed bone and BioOss® graft existed together until 6 months after bone grafting. Yildirim et al. [23] also confirmed that there is residual graft till 6-7 months after the surgery. Most of the mineral particles are included into newly formed bone.

To have a barrier against connective tissue is one of the most important factors of GBR in dehiscence-type defect. These soft tissue cells migrate and proliferate faster than bone forming cells [24,25]. Therefore, barrier membranes are used for occluding defect area directly from connective tissues in GBR [14,26,27]. Barrier membranes are divided into resorbable and non-resorbable membrane with unique advantages and disadvantages. Resorbable membrane has an advantage of no additional surgery required for eliminating membrane later; whereas, the drawback is lack of space maintenance due to lack of rigidity, which is one of the important role of barrier membrane. On the other hand, non-resorbable membrane has enough barrier role to occlude connective tissues and effectively maintains space for bone regeneration, but requires secondary surgery for removal. Both types of barrier membrane cause associated complications from membrane exposure. Chiapasco and Zaniboni [6] reviewed the literature on GBR and suggested that associated complications from non-resorbable and resorbable membrane occurred at the rate of 20% and 5% respectively. Furthermore, barrier membranes are expensive and difficult to handle [5]. In the present study, xenograft materials, which

Figure 5. A measurement of HBW and VBH for BBA technique group was done with the analysis program. The graph of left side has an X and Y-axis that are a distance (per pixel) and signal intensity, respectively. HBW: horizontal bone width, VBH: vertical bone height, BBA: bilayer bone augmentation

Table 1. Specific group wise trends in the 3 parameters through trend analysis (mean±variation)

	Control	AB	BBA	XB	<i>p</i> for trend
Numbers	4	5	4	6	
Bone volume (BV, mm ³)	8.1 ± 5.99	7.01 ± 5.61	10.42 ± 3.5	11.08 ± 3.2	0.1727
Horizontal bone width (HBW, mm)	1.03 ± 0.44	1.06 ± 0.23	1.3 ± 0.26	1.33 ± 0.25	0.0614
Vertical bone height (VBH, mm)	4.67 ± 0.13	4.74 ± 0.17	4.85 ± 0.12	4.88 ± 0.1	0.0112*

General Linear Model (**p* for trend <0.05). AB: autogenous bone graft, XB: xenograft, BBA: bilayer bone augmentation

have a comparatively slow resorption rate with better space maintenance, were applied as an outer layer as a potential barrier membrane substitute. If the outer xenograft layer is adequately replaced with the barrier member, the associated complications, additional surgery requirement and cost would be resolved. In the present study, each parameter value was highest in the xenograft group followed by the BBA technique group. Thus, the xenograft has superiority in terms of BV and space maintained for bone formation in dehiscence-type defect.

Autogenous bone material is an exclusive first choice candidate for osteogenesis in GBR, but requires a donor collection site and furthermore results in postoperative morbidity. In addition, autogenous bone can be continuously resorbed unless additional treatments are implemented to maintain a proper support during the bone remodeling process [28,29]. There are some differences in repair processes between cortical bone and cancellous bone in the autogenous bone graft. Lyford et al. [30] described that re-vascularization of cancellous bone graft is earlier than that of cortical bone graft and the repair process of cortical bone graft remains a mixture of necrotic and viable bone for a prolonged period. Moreover, they pointed out that the mechanical strength of cortical bone graft is weakened, whereas the mechanical strength of cancellous bone graft tends to be strengthened. In the present study, the particulated corticocancellous bone was collected from the mandibular canine position of dog. However, because of donor site position, the collected autograft was mainly composed of cortical bone. The BV, HBW, and VBH parameters were lower in the AB and BBA groups than the XB group. This was possibly due to the less than expected osteogenic ability of autogenous bone col-

lected in the present study. However, no significant between group differences were observed in BV, HBW and VBH.

The results of the present study showed that the BBA group had no significant differences statistically, as compared with the control, AB and XB group; however, it clearly showed higher value of BV, VBH, HBW than control and AB group. VBH tended to be statistically higher in sequence of control, AB, BBA and XB group. Collectively, these results confirmed that the xenograft and BBA technique group are comparatively superior in terms of stability in bone graft area. Values of the 3 parameters for BBA technique group were slightly lower than the xenograft group. It was presumed that there was resorption of the inner autograft layer during the process of remodeling. Although additional studies are needed on barrier membrane, the xenograft layer on BBA group played the role of barrier membrane with space maintenance on comparing each parameter between AB group and BBA technique group.

In the present study, all 3 parameters of BV, HBW, VBH at the area of bone graft were measured and analyzed by micro CT. Currently, micro CT is generally used for many studies on bone in the dental implant area [31-33]. Conventional histomorphometry provides unique information on cell and tissue, but it is difficult to use specimens for other examinations (ex. removal of torque force) due to preparation of sections [34]. Micro CT allows observation of bone tissue in 3D images, as well as quantitative analysis on various sections that is not possible on histomorphometric analysis [35]. Müller [36] has also reported that micro CT is recommended for analysis and 3D evaluation of corticocancellous bone, when compared with conventional histomorphometry. Thus, micro CT has more advan-

Figure 6. Results of micro CT scanning analysis of 3 parameter (◇: mean). AB: autogenous bone graft, XB: xenograft, BBA: bilayer bone augmentation

Table 2. Statistical comparison of the values between groups

	Control-AB	Control-XB	Control-BBA	AB-XB	AB-BBA	XB-BBA
BV (mm ³)	0.9848	0.7529	0.8919	0.4894	0.6967	0.996
HBW (mm)	0.9979	0.4115	0.5695	0.4616	0.6334	0.9986
VBH (mm)	0.8684	0.1107	0.2632	0.3301	0.6049	0.985

General linear model ($p < 0.05$). AB: autogenous bone graft, XB: xenograft, BBA: bilayer bone augmentation

tages of measuring bone product and various values in a rapid, accurate, and 3 dimensional manner.

Conclusions

The results of this preliminary study showed the usefulness of the BBA technique with mechanical support for prolonged space maintenance of xenogenic bone for the treatment of dehiscence-type defects around implants. However, further studies with a larger sample size are required to confirm these results. Additional experimental studies on bone graft with another type of bone substitute are also necessary.

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

The study was approved by the Committee on the Use and Care of Animals and the IRB of the Catholic University of Korea (2013-0079-01).

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