Effect of Bone Mineral with or without Collagen Membrane in Ridge Dehiscence Defects Following Premolar Extraction

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[1]College of Veterinary Medicine, Chungbuk National University, Cheongju;
[2]Department of Veterinary Nurse and Pet Science, Seojeong College, Yangju;
[3]College of Veterinary Medicine, Chonnam National University, Gwangju;
[4]College of Veterinary Medicine, Kangwon National University, Chunchon, Republic of Korea

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[3]*College of Veterinary Medicine, Chonnam National University, Gwangju;*
[4]*College of Veterinary Medicine, Kangwon National University, Chunchon, Republic of Korea*

Abstract. *Background: The purpose of this investigation was to evaluate the regenerative response to deproteinized porous bovine bone mineral (BM) when used alone or in combination with a bioresorbable porcine-derived bilayer collagen membrane (CM) for alveolar ridge augmentation in dogs. Materials and Methods: The mandibular premolars were extracted unilaterally and three ridge defects were induced in six mongrel dogs. Each defect site was randomly assigned to one of the following treatment groups: BM alone (group A), BM in combination with CM (group B), or neither membrane nor bone graft, which served as a control (group C). No adverse events occurred during the experimental period. Dental computed tomography (CT) scans were taken after postoperative periods of 8 and 16 weeks. Results: The percentage of CT-derived bone density in groups A and B was significantly different from that of group C ($p<0.01$) at 8 and 16 weeks. The percentage of CT-derived bone density of the dogs in Group B was significantly higher than that of those in group A at 8 and 16 weeks ($p<0.01$). Gross evaluation of the 3-dimensional CT reconstruction image of the canine mandibles after 16 weeks of implantation showed that group B had the greatest amount of bone augmentation and excellent thickness of the buccal aspect of the alveolar ridge. Conclusion: These results suggest that BM leads to more successful bone regeneration for guided bone regeneration procedures, especially in conjunction with the use of a CM as a barrier in order to promote the regeneration of canine alveolar ridge defects.*

Correspondence to: Seok Hwa Choi, DVM, Ph.D., Department of Veterinary Surgery, College of Veterinary Medicine, Chungbuk National University, Cheongju, 361-763, Republic of Korea. Tel: +82 43 2613144, Fax: +82 43 2613320, e-mail: shchoi@cbu.ac.kr

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A variety of techniques have been developed to promote bone formation in residual defects, such as dehiscences, fenestrations and infraosseous or supracrestal deficiencies in the bone regenerative procedure (1, 2). These techniques include the use of resorbable or nonresorbable barrier membranes, autogeneous or allogenic bone grafts, and nonbone allografts (3). Deproteinized porous bovine bone mineral (BM) has recently been investigated and studies have shown that it may be considered to be both biocompatible and osteoconductive, and therefore can be used to provide a scaffold for new bone growth (4). Studies in animal models have shown that BM is capable of becoming well-vascularized and integrated with the new host bone (5, 6). Berglundh and Lindhe (7) reported the development of a new connective tissue attachment with bone regeneration and new cementum deposition for the treatment of canine infrabony defects.

Previous reports have demonstrated the enhanced effect of combining a bioresorbable porcine-derived bilayer collagen membrane (CM) with a bovine-derived xenograft to form a physical barrier. Clergeau *et al*. (8) reported significant bone regeneration in experimental canine intrabony defects by using anorganic bone plus collagen in comparison to plain flap curettage-treated controls. Xenogeneic natural BM in combination with CM was found to elicit up to 7.6 mm of new attachment formation (9).

Computed tomography (CT) scanning has been shown to be helpful in the postoperative evaluation of bone grafts in maxillofacial reconstruction, including implant installation (10). Recently, techniques using CT have provided a method not only for assessing the infrastructure, but also for quantifying the bone mineralization defined by quantitative CT (11, 12). Although CT is mostly used to determine bone quantity, it was recently suggested that CT may have the potential to measure "bone quality," defined as the mean value of Hounsfield units (HU), and thus may be used to avoid placing the implant into the parts of the bone with the very poorest qualities where failure is most likely to occur (13).

While this is encouraging, there has been limited investigation into whether or not the anorganic xenograft is associated with absorbable barrier membranes, based on image analysis from CT in the alveolar ridge augmentation. The aim of this study was to evaluate the bone regenerative outcome of anorganic bovine BM combined with or without a resorbable CM as a barrier based on the changes in CT-derived density and 3-dimensional (3D) morphology of bone in the treatment of surgically created ridge dehiscence defects following extractions in dogs.

Materials and Methods

Animals. Six adult male mongrel dogs without general or oral health problems, 18 to 24 months old weighing 10 to 15 kg, were used for this experiment. During the entire study period, all dogs were fed a soft diet (Merry dog®; Nestle Purina Co., Korea) and water *ad libitum*. This protocol was approved by the Animal Care Committee of Chungbuk National University.

Surgical procedures. Prior to the regenerative procedure, all teeth were scaled and cleaned, and antibiotics (Procaine penicillin G 20,000 I.U./kg, Tardomyocel®; Bayer, Korea) were administered intramuscularly. The dogs were premedicated with the subcutaneous administration of 90.04 mg/kg atropine sulfate (Kwang-Myung Pharm., Korea) and were sedated subcutaneously with 2% xylazine (2 mg/kg, Rompun®; Bayer, Korea). Anesthesia was induced and maintained with tiletamine and zolazepam (7.5 mg/kg, Zoletil®; Virvac, Korea). To control bleeding and ensure profound anesthesia, 2% lidocaine HCl containing epinephrine at a dilution of 1:100,000 were administered by infiltration at the buccal aspect of the lower jaw.

Buccal and lingual intrasulcular incisions around the necks of the teeth were made from the mesial of the first premolar to the distal of the first molar. Vertical incisions were made to allow for adequate access to the surgical site and to achieve complete coverage of the membrane with the mucoperiosteal flap. Full-thickness mucoperiosteal flaps were carefully reflected to expose the underlying bone. The left mandibular second, third, and fourth premolars were atraumatically extracted using Cowhorn forceps (No. 16) according to the extraction forceps method, while taking great care to avoid root and cortical bone fracture.

The interradicular bone and buccal cortical plate of the extraction sockets were surgically reduced in width and height using a No. 2 round bur with a high-speed handpiece (Dentalaire Co., USA) under copious irrigation with sterile saline to produce a total of three buccal dehiscence defects. Periodontal probes (CP-12, Hu-Friedy Co., USA) were used to measure the defects. These rectangular bone defects were approximately 6 to 8 mm in height, 2 to 3 mm in depth, and 8 to 9 mm in width. Although individual differences in the dimensions of the alveolar ridge did not allow for perfect standardization of the defects, special effort was made to keep the defect dimensions constant.

The extraction socket defects were assigned to one of the following three treatments: one site was treated with a BM graft alone (Bio-Oss®; Geistlich Biomaterals, Wolhusen, Switzerland), one site received a BM graft plus a CM (Bio-Gide®; Geistlich Biomaterals, Wolhusen, Switzerland), and one site received neither BM nor CM and served as a negative control. The treatment locations were randomly chosen.

A total of 0.5 g of BM cancellous granules previously mixed with sterile saline was applied to each socket dehiscence defect site. We tried to restore as much of the alveolar ridge morphology as possible, with the intension of a slight overfilling. The CM was trimmed to the required size and then adapted to the shape of the individual defect so that the membrane overlapped the walls of the defect by at least 2 mm in order to achieve complete coverage of the bone and to prevent the ingrowth of soft connective tissue. The membrane absorbed the blood and easily covered and adhered to the underlying bone by applying gentle compression with a gauge. After covering the defect and bone graft, the membrane was sutured to the underside of the flaps to prevent displacement with interrupted sutures (5-0 Surgifit; AILEE Co., Korea). Finally, the mucogingival flaps were closed with sutures (4-0 Surgifit) and left as tension free as possible to ensure coverage the membrane margins. Following all surgical procedures, the dogs were medicated with antibiotics for infection control (Procaine penicillin G, 20,000 I.U./kg, *i.m.* every 24h for 6 days; Tardomyocel®; Bayer Co.) and with steroids for anti-inflammatory pain control (dexamethasone, 2 mg/dog, *i.m. s.i.d*, day 1 and 4; Voren®; Boehringer Ingelheim Co.). The oral cavities were rinsed daily with 0.12% chlorhexidine-digluconate for plaque control during the first two postoperative weeks.

Dental computed tomography imaging. Dental CT scans of the head were obtained under general anesthesia in the same manner as in the surgical procedures 8 and 16 weeks post-operatively. The dogs were positioned in ventro-dorsal recumbency on the cradle of a CT scanner, and the position of their heads was adjusted for symmetry. High-resolution transverse imaging of the mandible of each dog was obtained with a conventional CT scanner (CT-HiSpeed Advantage™, USA). Images were taken contiguously at 1-mm intervals with a resolution close to 0.25 mm/pixel at 130 kVp and 20 mAs using a standard dental CT investigation protocol. The thin transverse image data obtained from the mandible were reformatted using special software (10DR® Implant Service Co., Korea) to automatically produce cross-sectional images, curved linear panoramic images and a 3D reconstruction image every 1 to 2 mm around the dental arch. The CT images were reconstructed to define the bone contours. The special software displays the CT number or HU over the selected regions of interest (ROI), thereby estimating the bone density at specific sites for the evaluation of defective extraction sockets filled by grafted mineral particles postoperatively. The average pixel densities of a 5 mm^2 ROI area centered over the crestal areas of all extraction socket defects on a panoramic projection image that was reconstructed from panoramic images were expressed as a percentage in comparison with the intact adjacent alveolar bone (Figure 1).

Statistical analysis. The differences between the measured bone density values 8 and 16 weeks postoperatively in the three groups were evaluated using one-way analysis of variance (one-way ANOVA) with the Tukey's test. The data were presented as the mean±standard deviation (SD) and $p<0.05$ was considered statistically significant.

Results

All of the dogs recovered well from the anesthesia and the surgical interventions, and they all behaved normally throughout the remainder of the study. There were no postoperative complications, such as signs of infection, abscesses or allergic reactions, during the entire period of the experiment.

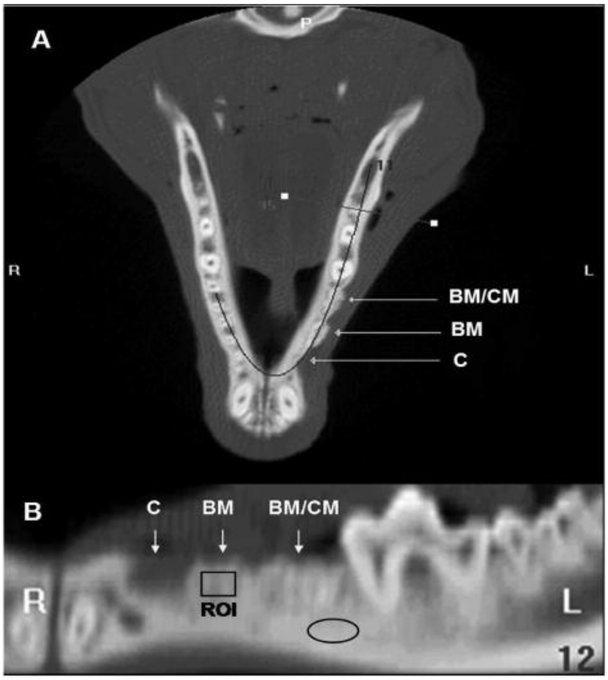

Figure 1. *View of the display of the software program showed defective extraction sites (arrows) filled by grafted mineral particles on the CT slice at 8 weeks post-operation. A, One of the transverse images was selected as the reference transverse slice image. One arch to the center plane was drawn, and this was taken as a reference point when carrying out the series of panoramic images. B, A "panoramic projection" image was reconstructed from serial panoramic images. The average pixel densities of a 5 mm^2 ROI area (indicated as a square) centered over the crestal areas of all extraction socket defects on the panoramic projection image was divided by that of the intact adjacent alveolar bone (indicated as circle) and expressed as a percentage.*

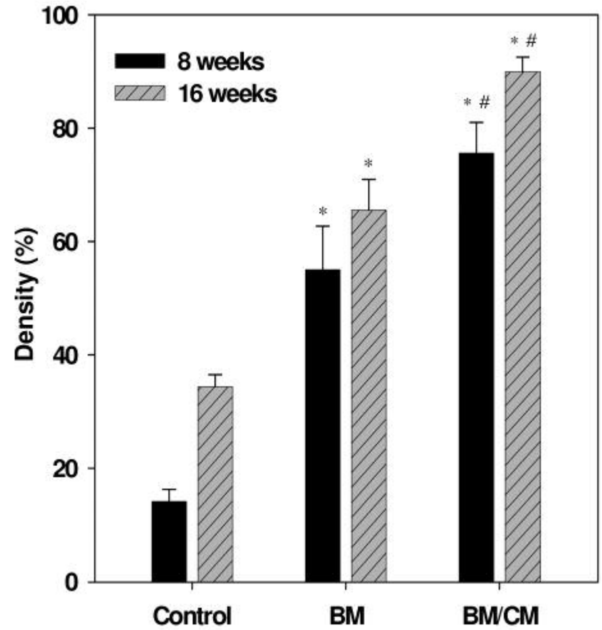

Figure 2. *Bone density measured by dental CT at 8 and 16 weeks postoperatively in each group (%). Symbols indicate significant differences (p<0.01) determined by Tukey's test; *compared with control group; #compared with BM-treated group alone.*

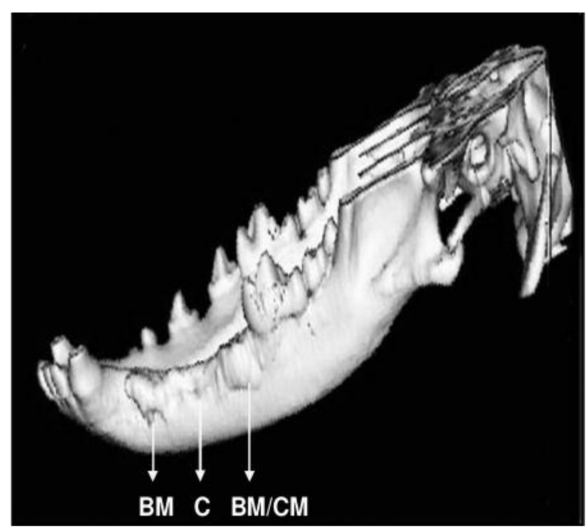

Figure 3. *Gross evaluation of the 3D-CT reconstruction of the CT scan after 16 weeks of implantation clearly indicated more osseous fill buccally in both BM- and BM/CM- treated sites than that in the control group, which revealed alveolar ridge collapse due to inadequate osseous fill. The CM enhanced bone regeneration, especially in conjunction with the use of a supporting graft material.*

The results of CT-derived densitometry in each group are shown against the time after the operation in Figure 2. The data show a general increase in bone density for each treatment group over the 16 weeks with time. The bone densities as compared to intact adjacent alveolar bone were 55.0±7.6% and 75.6±5.4% at 8 weeks, and 65.5±5.5% and 89.9±2.6% at 16 weeks, for the BM- and BM/CM-treated groups, respectively. In comparison, the bone density of the control was 14.2±2.1% and 34.3±2.2% at 8 and 16 weeks, respectively. At 8 and 16 weeks postoperatively, the mean bone density values in treated groups were significantly higher than that of the control group ($p<0.01$). At 8 and 16 weeks postoperatively, the mean bone density value in the BM/CM-treated group was significantly higher ($p<0.01$) than that of the BM-treated group.

Three-dimensional reconstructions of the bone graft at 16 weeks postoperatively, which were obtained by elaborating the tomographic sections, also showed a difference in outcome of bone regeneration following ridge augmentation between the control and experimental sites, as shown in Figure 3. In the present study, it was significant that increases in bone height apparently occurred at the grafting sites and that the ridge height response demonstrated that bone formation equaled or exceeded a complete fill of the extraction socket defects in the grafting sites. In particular, areas implanted with BM combined with CM provided the greatest amount of bone augmentation and excellent thickness of the buccal aspect of the alveolar ridge. Satisfactory contour was also established in areas of BM without CM. In contrast, the control defects still exhibited incomplete bony thickness and poor ridge formation buccally.

Discussion

The ultimate goal of periodontal therapy is the control of periodontal infection and the regeneration of bone, cementum, and periodontal ligament loss due to periodontitis (14). The therapy of guided bone regeneration (GBR) using barrier membranes, which has been frequently combined with the application of autogenous bone grafts or a bone substitute, is a new technique that evolved from the guided tissue regeneration (GTR) procedure for the reconstruction of lost periodontium (15). Quiñones and Caffesse (16) clarified that the term GTR applied to procedures aiming at either the regeneration of lost periodontal structures (*i.e.* cementum, periodontal ligament and alveolar bone) resulting from periodontitis, and those with the goal of regenerating the alveolar bone alone, such as bone augmentation, prior to or in association with the placement of osseointegrated dental implants (*i.e.* GBR).

CM is a bioresorbable collagen barrier made of fiber protein, which consists of hydroxyproline with the structure of a triple helix. Collagen fibers provide structural elasticity during the crystalline phase of bone regeneration (17). The properties of collagen ensure optimal tissue integration and adequate wound healing (18). In the present study, the fact that no adverse reactions, such as allergy or abscess after membrane implantation, were observed also supports the biocompatibility of the membrane material. In addition, the good tissue integration properties of the membrane material used in this study and the lack of postoperative complications may also be responsible for the significant gain in regenerative bone fill.

The critical importance of space maintenance is one of the main problems in GTR and GBR procedures (19). In fact, the collagen membrane itself cannot contain a cavity because it lacks the necessary stiffness to maintain sufficient space under the membrane to fill the defect. It is therefore desirable to use a grafting material when applying resorbable membranes (15, 17). Porous BM, which is a xenographic bone graft material, is obtained by extracting all of the protein from bovine cancellous or cortical bone (20). Over time, the graft undergoes physiological remodeling and becomes incorporated into the surrounding bone (21). The ability of the materials to enhance bone regeneration in animal experiments and human clinical studies has been reported (7, 22). However, although clinical success has been established, it is proposed that the addition of a physical barrier assists in graft containment and wound stabilization (23), which may in turn enhance the regenerative effects of the bone graft alone. The enhancement of the clinical parameters in both intrabony and furcation defects has been shown with the combined use of xenograft and a membrane in comparison to therapy alone (24, 25). Camelo *et al.* (9) histologically demonstrated new cementum, periodontal ligament and bone formation in human periodontal defects, which were noted by a probing depth reduction of 5 mm and clinical attachment level gain of 7.0 mm in combined treatment cases.

Currently, the progress of healing is primarily assessed on the basis of radiographic changes in the grafted bone and also on the basis of the response of the grafted tissue to spontaneous migration or orthodontic movement of teeth into the former cleft site (26). Although bone remodeling taking place at the site of bone grafting and 2-dimensional changes can be evaluated by conventional radiographs (27), a minimum bone mass loss of 30%, and sometimes as much as 50~60% is needed before significant osteopenia can be detected radiographically, and conventional visual analysis techniques are not precise enough to detect subtle changes in bone density (28). There are many radiographic procedures for the measurement of bone volume (29, 30); however, measures that quantify bone quality (31) with rather crude grading methods (29) may be used but will hardly reflect the exact situation of the bone at a planned surgical site. CT scans offer the best radiological method for morphological and qualitative analysis of bone. An additional advantage of measurement by CT scan is that bone density can also be calculated using CT data (32).

Decreases in bone density result from the decalcification and reduction of the trabecular bone. Therefore, since bone density directly correlates with bone quality, it is important to obtain preoperative information on bone density (13). The densitometric results of this study demonstrated that the healing of the bone in extraction socket defects using BM with or without CM is expressed as an increase in osteoid formation and mineralization, resulting in a higher bone mineral density in the bone image. The hard tissue results showed that there was a greater improvement in the regenerative bone response in deficient extraction sockets treated with a combination of BM and CM than in those treated with BM placement alone. The Tukey test results showed a significant difference ($p<0.01$) in mean bone density changes between sites treated with and without membranes. Lower cancellous bone density in ridge defects filling with BM particles alone may result in compromised osteogenesis or excessive resorption, in comparison with higher density bone, in combination treatment with BM and CM, thereby upsetting osseous healing.

Resorption causes not only reduction in bone volume but also decalcification and reduction in the trabecular bone (13). This effect can be measured as a decrease in bone density (33). However, there is conflicting evidence in the literature as to the resorption of BM particles. Some studies report resorption (34, 35) while others report lack of resorption (36, 37). In the present study, the BM grafts showed no decrease in bone density. During the 16-week postoperative period, the density in all of the experimental grafting sites showed an increasing pattern. This suggests that the increase in bone density at defect sites with the use of BM with or without CM observed in this study was possibly caused by the remodeling processes rather than by the increased resorption of the grafts.

Three-dimensional CT scans have been used to assess the volume of bone grafts in relation to subtraction radiography (38). Three-dimensional analysis has been recommended for precise diagnosis and is currently being used in the area of craniofacial morphology (39). This nondestructive technique provides an opportunity for the very precise evaluation of hard tissues at various locations throughout a treated site (40). In the gross evaluation of the 3D-CT reconstruction of the canine mandibles after 16 weeks of implantation, areas implanted with BM in combination with CM exhibited the greatest amount of bone augmentation and showed excellent thickness of the buccal aspect of the alveolar ridge, whereas poor bone formation was observed at the buccal aspect of the defect site in the control animals, which had a moderate ridge collapse with a knife-edge ridge appearance. These results suggest that the porous BM may have served as an osteoconductive scaffold, not only to support the membrane and preserve space, but also to facilitate better and faster bone healing of bone defects.

The advantage of establishing initial blood clot stabilization with a BM in combination with a CM is that it is less likely that there will dead space under the membrane (41). Moreover, the addition of a physical barrier assists in graft containment and wound stabilization with selective cell repopulation from the adjacent bone, while excluding the gingival corium cells that usually migrate to the wound healing site first (42). Furthermore, it appeared that BM, which was used as a space maintenance material beneath the membrane, served as a matrix for angiogenesis and osteogenesis, thus enhancing the regeneration process (21).

Based on the results of the present study, we conclude that the use of porous xenographic bone grafts in combination with bioresorbable collagen barrier membranes in the GBR procedure can enhance successful bone regeneration in the treatment of canine alveolar ridge defects.

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