Guided Bone Regeneration with the Combined Use of Resorbable Membranes and Autogenous Drilling Dust or Xenografts for the Treatment of Dehiscence-type Defects Around Implants: An Experimental Study in Dogs

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Purpose: *This study aimed to measure the numbers of viable bone cells present in autogenous drilling dust (ADD) and mandibular particulated bone (MPB) and to histomorphometrically compare the effects of the combined use of resorbable membranes and ADD or xenografts for the treatment of dehiscence-type defects around implants.* ***Materials and Methods:*** *The left mandibular premolars were extracted from 4 adult beagle dogs. After a 3-month healing period, 4 standardized bone defects were prepared on each mandible, and 1 implant was placed per defect. The 4 sites in each dog were allocated to 4 different treatment groups: 1 site received ADD alone (ADD); 1 site received a Cytoplast membrane supported by ADD (ADD+CP); 1 site received BioCera alone (BC); and the final site received a Cytoplast membrane supported by BioCera (BC+CP). Each animal received a series of 3 bone labels. Three months following these regenerative surgeries, animals were sacrificed and histomorphometric examinations were carried out. In addition, in 3 of the 4 dogs at the time of regenerative surgery, ADD was obtained using implant drills (group 1), MPB was obtained using a fissure bur and rongeur (group 2), and 1.0 cm^3 of each was then cultured. Cultured cell counts and osteocalcin synthesis analysis using reverse transcription-polymerase chain reaction were performed on cells from these 2 groups at 4 and 9 weeks after regenerative surgery. Alkaline phosphatase activity (ALP) was measured at 9 weeks in both groups.* ***Results:*** *MPB revealed greater cell counts than ADD after 4 and 9 weeks. Cells stained positively for ALP and osteocalcin in both groups. Fluorescence microscopy showed 22.4% bone formation with ADD+CP, 17.8% with BC+CP, 13.1% with ADD, and 6.4% with BC at 8 weeks. Bone regeneration heights were 2.0 mm with AD, 1.9 mm with ADD+CP, 1.7 mm with BC+CP, and 1.3 mm with BC. Bone regeneration areas measured 1.0 mm^2 with ADD, 0.9 mm^2 with ADD+CP, 0.6 mm^2 with BC+CP, and 0.3 mm^2 with BC. Bone-to-implant contacts were 53.1% with ADD, 46.6% with ADD+CP, 44.1% with BC, and 33.7% with BC+CP.* ***Conclusions:*** *ADD appears to be a useful material for closing dehiscence-type defects, and the use of a membrane was not found to affect bone formation during the treatment of dehiscence-type defects around implants in this study. However, larger studies are needed before fully endorsing its widespread use.* Int J Oral Maxillofac Implants 2008;23:1089–1094

Key words: *alkaline phosphatase, autogenous drilling dust, dehiscence, osteocalcin*

One of the key factors for achieving osseointegration is the presence of an adequate osseous volume. In patients with an inadequate osseous width or height, bone augmentation using guided bone regeneration (GBR) may be applied using either a simultaneous or staged approach.[1] Autogenous bone grafting remains the gold standard for bone regeneration, since autogenous bone grafts are both osteoconductive and osteoinductive, while providing optimal conditions for the penetration of blood vessels and migration of cells with osteogenic potential. However, autogenic bone grafting usually requires a

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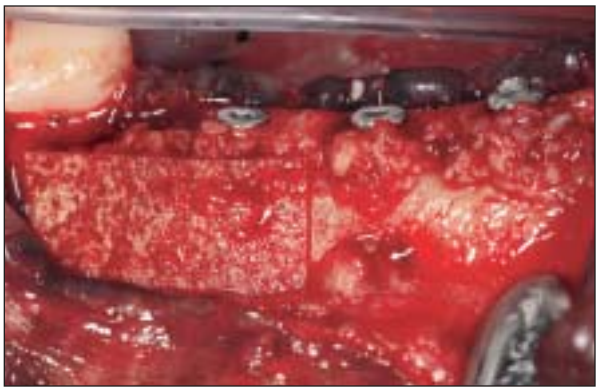


Fig 1 Alveolar crest showing the 4 sites undergoing regenerative procedures.

second operation site and may cause various degrees of morbidity in the donor area. However, these problems can be overcome if GBR using autogenous drilling dust (ADD) is performed.

The purpose of this study was to measure the amounts of viable bone cells present in ADD and mandibular particulated bone (MPB) and to histomorphometrically compare the effects of the combined use of resorbable membranes and ADD or xenografts for the treatment of dehiscence-type defects around implants.

MATERIALS AND METHODS

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, Republic of Korea.

Surgical Procedures

Four adult beagle dogs weighing more than 12 kg each were used in this study. All dogs were checked for health status by a veterinarian before the study. An accommodation time of 2 weeks prior to any kind of surgery was allowed. The first, second, third, and fourth mandibular left premolars were extracted from each dog. All surgical procedures were performed under general anesthesia using preanesthesic sedation with ketamine (Ketatar, Yuhan, Seoul, Republic of Korea), followed by intubation and maintenance with a Harvard respiration pump for the duration of surgery. After 3 months of healing following tooth extraction, defects were created, titanium implants were placed, and regenerative surgery was performed. Before implant placement, 4 rectangular bone defects measuring 4 mm in height from the crestal bone, 3 mm in width mesiodistally, and 4 mm in depth from the surface of the buccal bone were surgically created. Sixteen implants (PITT-EASY BIO-OSS Implants, Oraltronics, Bremen, Germany) with a diameter of 3.25 mm and a length of 10 mm were then placed. The implants were placed in such a manner that the upper surfaces were in line with the alveolar crests. Pretreatment dehiscence Class I defects, as described by Tinti and Parma-Benfenati,[2] were measured from the upper end region to the base of the buccal defects using a standard periodontal probe. The 4 sites in each dog were allocated to 4 different treatment groups: 1 site received ADD alone (ADD); 1 site received a Cytoplast membrane (Oraltronics) supported by ADD (ADD+CP); 1 site received BioCera (bovine bone coated with calcium-phosphate nanocrystal thin film; OCT, Cheonan, Republic of Korea) alone (BC); and the remaining site received a Cytoplast membrane supported by BioCera (BC+CP) (Fig 1). The membrane was stabilized before sutures were placed. Animals were maintained on a soft diet from regenerative surgery until the end of the study.

Cell Culture and Cell Counts

In 3 of 4 dogs, ADD was obtained during drilling for implant placement (group 1), mandibular particulated bone (MPB) was obtained using a fissure bur and rongeur (group 2) from the mandibular area, and 1.0 cm^3 of each was then cultured. Bone chips were immersed in 10 mL of phosphate-buffered saline containing 0.06% Collagenase type I (Invitrogen, Carlsbad, CA, USA) for 12 hours at 37°C in a humidified 95% air/5% carbon dioxide (CO_2) atmosphere (CO_2 incubator, Forma Scientific, Marietta, OH, USA). After centrifugation for 10 minutes at 1,000 rpm, the pellets obtained were immersed in Dulbecco's Modified Eagle's Medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Gibco/BRL, Grand Island, NY, USA). Solutions were filtered through 70-μm cell strainers (BD Bioscience, Bedford, MA, USA), and after filtration, cells were immersed in the same culture medium and incubated at 37°C in 5% CO_2 for 4 or 9 weeks, when the cells were released using trypsin-ethylenediaminetetraacetic acid (Gibco/BRL) and then counted.

Alkaline Phosphatase Activities of Cultured MPB and ADD

Alkaline Phosphatase (ALP) activities were measured using Sigma Diagnostic Kits (86-R, Sigma-Aldrich, St Louis, MO, USA) 9 weeks after regenerative surgery.

Osteocalcin Synthesis of Cultured MPB and ADD

Cellular RNA was extracted using RNeasy Mini Kits (Qiagrnmax-volmer-strabe, Hilden, Germany), and osteocalcin expressions were estimated using

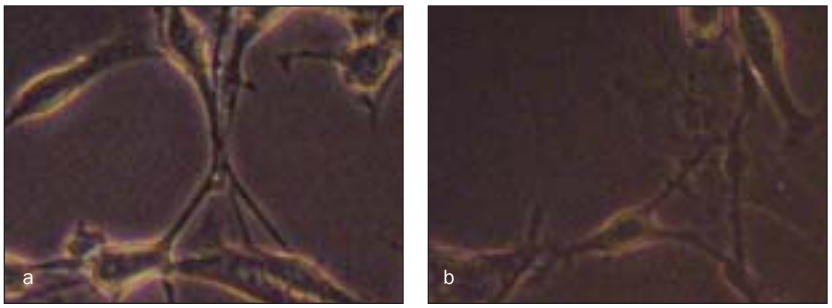


Fig 2 Photomicrographs (× 200) showing the osteoblasts after a 4-week culture period in group 1 *(a)* and group 2 *(b)*.

Table 1 Cell Counts in Groups 1 and 2 After a 4-Week Culture Period (Cells/mL)

	Group 1	Group 2
Dog 1	1.4×10^6	2.4×10^6
Dog 2	1.6×10^6	2.3×10^6
Dog 3	1.5×10^6	2.7×10^6
Mean	1.5×10^6	2.4×10^6

reverse transcription-polymerase chain reaction (RT-PCR) using osteocalcin PrimerMix Kits (Xenotech, Daejon, Republic of Korea) and PTC 200 (MJ Research, Ramsey, MN, USA) 4 and 9 weeks after regenerative surgery.

Bone Labeling

To assess patterns of osteogenesis and delineate the bases of the original defects, each animal received a series of 3 bone labels:

- Three days after regenerative surgery: OxyTetracycline hydrochloric acid (HCl) (Sigma-Aldrich), 20 mg/kg body weight intravenously
- Eight weeks after regenerative surgery: Alizarin red (Sigma-Aldrich), 20 mg/kg body weight intravenously
- Three days prior to sacrifice: OxyTetracycline HCl, 20 mg/kg body weight intravenously

Three months after regenerative surgery, animals were sacrificed and histomorphometric examinations were carried out.

Histologic Preparation and Histomorphometric Examinations

Jaws were dissected and block sections containing the experimental specimens were prepared. These specimens were placed in 4% paraformaldehyde for 1 week and then immersed in Villanueva bone-staining solution for 1 week at room temperature. Specimens were dehydrated using a graded ethanol series before being embedded in methyl methacrylate resin. Cross sections of approximately 300 μm were cut in a buccolingual direction using a saw microtome (Maruto, Tokyo, Japan). One longitudinal histologic buccolingual section from each implant was evaluated under a fluorescence microscope (Fluorescence Attached Microscope, Olympus, Tokyo, Japan). Histologic evaluations around implants were performed at 40 × magnification. After picture digitization, the following histometric data were collected using a picture analysis program (Tomoro Scope-eye AUTO, Olympus, Seoul, Republic of Korea):

- Bone regeneration height: Linear distance between the defect base and the coronal level of regenerated bone
- Bone regeneration area: Total area of regenerated bone within the defect
- Bone-to-implant contact: Percentage of direct bone-to-implant contact related to the length of implant thread within regenerated bone

RESULTS

Cell Counts

After a 4-week culture period, the mean cell count was 2.4×10^4 cells/mL for MPB (group 2) and 1.5×10^4 cells/mL for ADD (group 1) (Figs 2a and 2b, Table 1). After a 9-week culture period, the mean cell count of group 2 was 2.5×10^6 cells/mL, while the mean cell count of group 1 was 1.7×10^6 cells/mL (Figs 3a and 3b, Table 2). Group 2 had greater cell counts than group 1 at both 4 and 9 weeks. Cell counts were greater at 9 weeks than at 4 weeks in both groups.

ALP Activity

Cells stained positively for ALP activity in groups 1 and 2 after 9 weeks (Figs 4a to 4f).

Osteocalcin Synthesis

Cells stained positively for osteocalcin after 4 and 9 weeks in groups 1 and 2 (Figs 5 and 6).

Histologic Findings and Histomorphometric Assessments

Based on fluorescence microscopy, the mean percentages of red bands were 22.4% with ADD+CP, 17.8% with BC+CP, 13.1% with ADD, and 6.4% with BC at 8 weeks. The mean percentages of yellow bands were 4% with ADD, 1.7% with BC+CP, 1.6% with ADD+CP, and 0.6% with BC (Table 3, Fig 7). His-

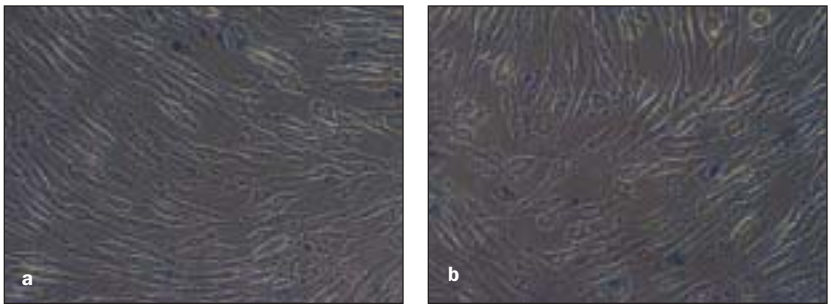


Table 2 Cell Counts in Groups 1 and 2 After a 9-Week Culture Period (Cells/mL)

	Group 1	Group 2
Dog 1	1.8×10^6	2.5×10^6
Dog 2	1.6×10^6	2.4×10^6
Dog 3	1.7×10^6	2.6×10^6
Mean	1.7×10^6	2.5×10^6

Fig 3 Photomicrographs (× 200) showing the osteoblasts after a 9-week culture period in group 1 *(a)* and group 2 *(b)*.

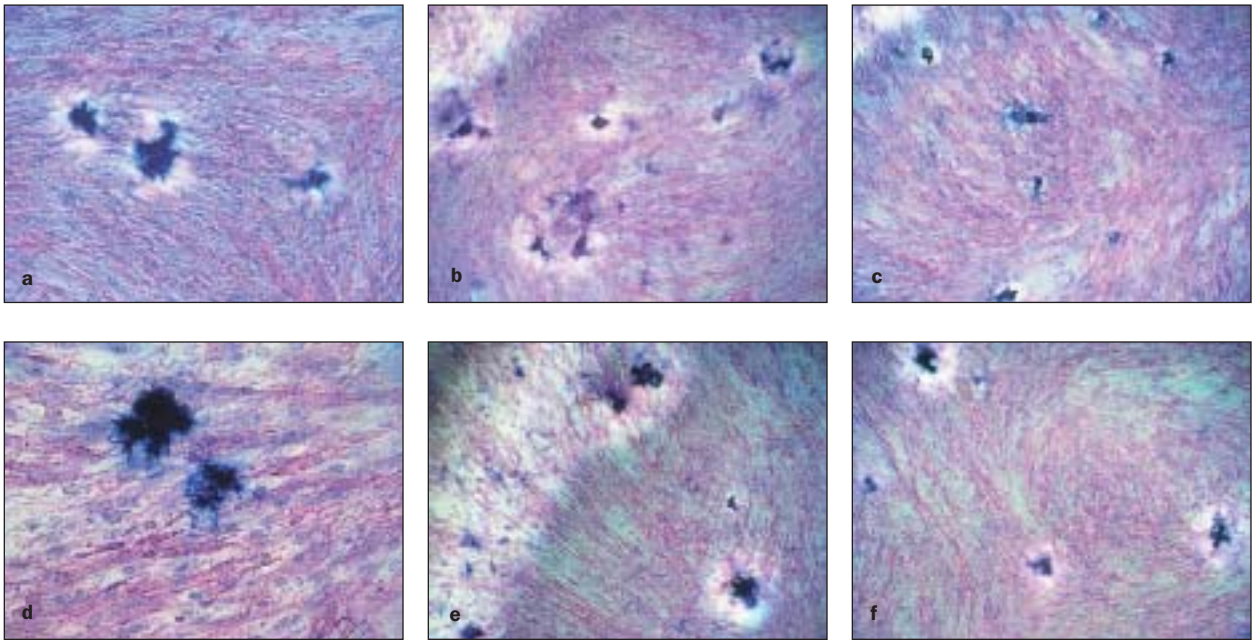
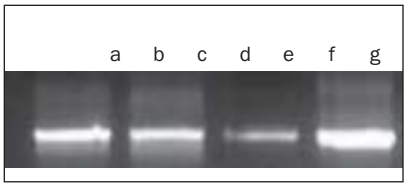


Fig 4 Alkaline phosphatase activity at week 9 (× 200) in group 1 *(a, b, c)* and group 2 *(d, e, f)*.



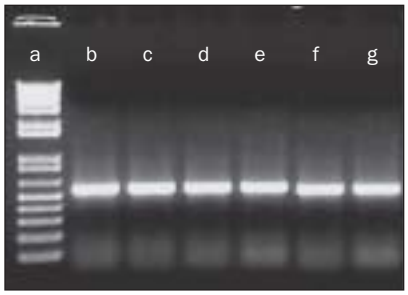


Fig 5 *(Left)* Osteocalcin expression at week 4: ADD *(a)* and MPB *(b)* in dog 1; ADD *(c)* and MPB *(d)* in dog 2; ADD *(e)* and MPB *(f)* in dog 3; 1-kb marker *(g)*.

Fig 6 *(Right)* Osteocalcin expression at week 9: 1-kb marker *(a)*; ADD *(b)* and MPB *(c)* in dog 1; ADD *(d)* and MPB *(e)* in dog 2; ADD *(f)* and MPB *(g)* in dog 3.

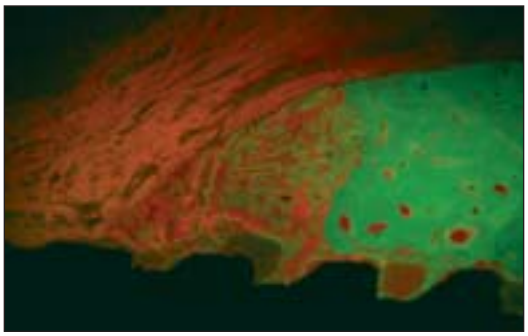


Fig 7 Fluorescence microscopy showing bone formation in the ADD+CP group 8 weeks after regenerative surgery.

Table 3 Mean Percentage of Red and Yellow Bands Present in Newly Formed Bone

Treatment	Red	Yellow
ADD	13.1	4.0
ADD+CP	22.4	1.6
BC	6.4	0.6
BC+CP	17.8	1.7

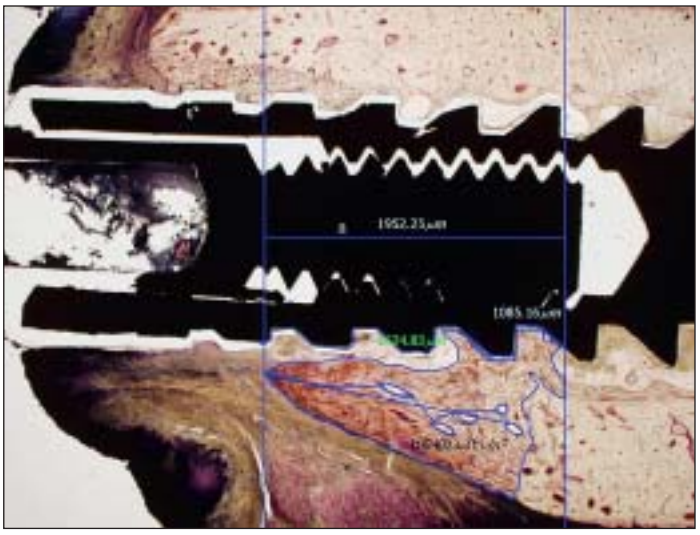


Fig 8 Histomorphometric analyses of bone formation in the ADD group: *(a)* bone regeneration height (μm); *(b)* bone regeneration area (μm^2).

tomorphometric assessments showed mean bone regeneration heights of 2.0 mm with ADD, 1.9 mm with ADD+CP, 1.7 mm with BC+CP, and 1.3 mm with BC. Mean bone regeneration areas were 1.0 mm^2 with ADD, 0.9 mm^2 with ADD+CP, 0.6 mm^2 with BC+CP, and 0.3 mm^2 with BC. The mean percentages of direct bone-to-implant contact were 53.1% with ADD, 46.6% with ADD+CP, 44.1% with BC, and 33.7% with BC+CP (Table 4, Fig 8).

Table 4 Results of Histomorphometric Analyses of Bone Formation (Mean ± SD)

Treatment	Bone regeneration height (mm)	Bone regeneration area (mm^2)	Bone-to-implant contact (%)
ADD	2.0 ± 0.1	1.0 ± 0.1	53.1 ± 16.7
ADD+CP	1.9 ± 0.9	0.9 ± 0.7	46.6 ± 24.9
BC	1.3 ± 0.8	0.3 ± 0.2	44.1 ± 18.5
BC+CP	1.7 ± 0.7	0.6 ± 0.5	33.7 ± 14.7

DISCUSSION

Following tooth loss, a natural process of alveolar bone resorption occurs. An insufficient amount of supporting bone may restrict the placement of endosseous oral implants. To prevent this from occurring, the GBR technique was developed to allow for the growth of bone tissue around implants placed in sites with insufficient bone volume.[3]

A number of efforts have been made to categorize bone defects for implant placement. Tinti and Parma-Benfenati[2] presented a classification of bone defects related to implant placement to help clinicians accurately discuss proposed treatment regimens and organize treatment for clinical correction. A further goal of their efforts involved the standardization of terminology to allow more accurate dental communication. Buccal dehiscence defects are one of the most commonly encountered problems in implant dentistry. When horizontal alveolar bony defects are present, bone regeneration prior to or during implant placement should be considered. In the present study, GBR was performed in Class I dehiscence-type defects as described by Tinti and Parma-Benfenati.[2]

Different authors have supported the use of various types of bone or bone substitute for GBR in dehiscence-type defects,[4,5] and a wide variety of materials have been applied in conjunction with GBR procedures. However, no single material has been demonstrated to be superior. Although autogenic bone has been claimed to be the standard material, well-controlled studies that have comprehensively evaluated all aspects are scarce.[6] Autogenic bone grafting usually requires a second operation site and may cause various degrees of morbidity in the donor area.[7] Donor site morbidity after the harvesting of autogenous bone cannot be neglected in the clinical setting; therefore, there is a constant need to develop and explore alternative techniques. The use

of ADD harvested during the drilling of the implant site presents one means of overcoming that problem. However, there have been concerns about the bone cells' viability because of overheating or infection during the drilling and bone harvesting procedures. Gruber et al[8] studied the proliferation and osteogenic differentiation of cells from cortical bone cylinders. Bone particles from a mill and drilling dust were harvested within 12 hours of death from 2 pigs. The authors suggested that these cells may contribute to bone regeneration following transplantation. However, drilling dust collected during cortical bone drilling was sucked into an aspirator and trapped using a filter in their study. The second operation site is inevitable in this dust-collecting method. Springer et al[9] measured the amounts of viable bone cells present in different types of particulated bone graft and reported that cells stained positively for osteocalcin and ALP in all types. In their study, drill sludge was obtained using a ball reamer, diamond ball, or implant drill. However, it could not be confirmed whether they followed the implant drilling procedure in the alveolar bone area.

In the present study, ADD was harvested from alveolar bone during the drilling of implant sites after 3 months of healing following tooth extraction. Cell outgrowth was observed, and cells stained positively for osteocalcin and ALP in MPB and ADD. Based on fluorescence microscopy and histomorphometric assessments, ADD showed better results than those of a xenograft, and the use of a membrane did not affect bone formation for the treatment of type I dehiscence defects around implants. These results show that ADD appears to be a useful material for closing dehiscence-type defects around implants. However, larger studies are needed to offer direct and conclusive data to fully support its widespread use.

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