A Comparative Study of Osseointegration of Avana Implants in a Demineralized Freeze-Dried Bone Alone or With Platelet-Rich Plasma

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**Purpose:** The purpose of this study was to assess the efficacy of demineralized bone powder (DBP) alone or combined in a mixture with platelet-rich plasma (PRP) used to enhance osseointegration of dental implants in a dog model.

**Materials and Methods:** Tissue integration was assessed using standard histomorphometric methods at 6 and 12 weeks after surgery. A total of 30 Avana dental implants (SooMin Synthesis Dental Materials Co, Busan, Korea) were inserted in the animals. They were self-tapping screw implants, 10 mm in length and 4 mm in diameter, made of commercially pure titanium. A titanium implant was then placed centrally in each defect. In each dog, the defects were treated with 1 of the following 3 treatment modalities: 1) no treatment (control), 2) grafting with DBP, or 3) grafting with DBP and PRP.

**Results:** Histologic analysis showed that all of the bone defects surrounding the implants that were treated with DBP, with and without PRP, were filled with new bone. The defects that were not treated (control) showed new bone formation only in the inferior threaded portion of the implants. Histomorphometric results revealed a higher percentage of bone contact with DBP and PRP compared with control and DBP.

**Conclusion:** These results suggested that bone defects around titanium implants can be treated successfully with DBP and that PRP may improve bone formation.

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Autogenous cancellous bone has been reported to be a satisfactory graft that can be placed into exposed implant surfaces. If the use of autogenous cancellous bone is limited due to a lack of host bone, alternatives such as allogeneic bone, xenogeneic bone, or alloplastic bone substitute have been used. Human demineralized freeze-dried bone powder (DBP) (Dem-
have resulted in the use of DBP as a graft material. DBP has been extensively used as regenerative material for treatment of bone defects adjacent to teeth with periodontal disease and for defects adjacent to dental implants. Studies relating to the treatment of periodontal defects have reported favorable decreases in probing depth, gains in clinical attachment levels, and gains in defect bone fill. To date, the clinical studies relating to the use of DBP adjacent to implants have reported coverage of exposed implant threads and expansion of deficient alveolar ridges. Previous studies have evaluated and compared the efficacy and degree of osseointegration of endosseous implants when placed in autogenous corticocancellous blocks and chips. However, there is a paucity of information on the minimum time needed to obtain a clinically integrated titanium implant when it is placed in a freeze-dried or demineralized bone graft. The main advantage of DBP is that it eliminates the need for a donor site. In addition, DBP is available in unlimited quantity. Its disadvantages are primarily those associated with the use of tissues from another individual.

The current widespread use of DBP is based on the purported osteoinductive ability of bone graft preparation. Demineralization of the graft exposes the bone-inductive proteins located in the bone matrix and in fact may activate them. Crude protein extracts from DBP contain immunoreactive bone morphogenetic protein (BMP) as well as other biologically active molecules. Demineralized bone has been studied extensively as an osteoinductive agent due to the presence of BMP. In contrast, other authors report that in animal experiments and clinical application, demineralized freeze-dried bone shows no evidence of osteoclastic resorption and no new formation of lamellar bone at its surface. Despite the apparent presence of BMP in DBP, direct clinical comparison of treatment success using mineralized freeze-dried bone allografts and DBP yielded similar results. Several possible explanations could account for the wide variation in clinical results when treatment with DBP is chosen. Potential causes might be insufficient quantities of bone inductive proteins, a lack of osteoinductive activity, delayed procurement of donor bone after death, improper storage temperatures, or other processing variables that may also play a significant role in the activity of the final DBP preparation. In addition, age, gender, and medical status of the donor before death may have a significant influence on osteogenic activity. The literature offers a conflicting evaluation of the efficacy of this material as a suitable graft for endosseous implants.

To our knowledge, the osteogenic capability of a combination of DBP and PRP used to fill a defect has not been previously reported. This study provides additional evidence in a dog model that DBP with or without platelet-rich plasma (PRP) supplementation can enhance osseointegration in the short term.

The purpose of this study was to assess the efficacy of DBPs alone or combined in a mixture with PRP used to enhance osseointegration of dental implants in a dog model.

**Materials and Methods**

**SURGICAL PROCEDURE**

This study was approved by the Animal Research Committee of Chosun University. Ten healthy, mature (1-year-old) male and female mongrel dogs were selected for the study. General anesthesia was induced by using pentobarbital and halothane. The surgical sites were shaved and prepared for a percutaneous sterile surgical procedure. A 2% lidocaine solution with 1:100,000 epinephrine was used to infiltrate the surgical site. Using a No. 15 Bard-Parker blade (Sheffield, England), a 6-cm incision was made over the iliac crest. The subcutaneous tissue, gluteal fat, and thoracolumbar fascia were incised. The crest and the wing of the ilium were exposed inferiorly for a distance of approximately 2 cm.

Three circular bone preparations measuring 4 mm apicocoronally and 6 mm mesiodistally and buccolingually were surgically prepared in iliac crest sites in each animal. A total of 30 Avana dental implants (SooMin Synthesis Dental Materials Co, Busan, Korea) were used as the experimental implants. The implants were self-tapping screw implants, 10 mm in length and 4 mm in diameter, of commercially pure titanium. A titanium implant was then placed centrally in each preparation in such a way that 3 threads were exposed and the cover screws were at the level of the intact proximal part of the crest. The defects were treated with 1 of the following 3 treatment modalities: 1) no treatment (control), 2) grafting with DBP, or 3) grafting with DBP and PRP.

**PRP PREPARATION PROCEDURE**

PRP was prepared following aseptic processing procedures. Blood was obtained several minutes before the administration of anesthesia. Then 10 mL of blood was drawn from each dog using 5-mL tubes that contained 10% trisodium citrate solution as an anticoagulant. The tubes were centrifuged at 1,000 rpm for 10 minutes at room temperature. The blood was thus separated into its 3 basic components: red blood cells, which appeared at the bottom of the tube; plasma rich in growth factors (PRGF), which appeared in the middle of the tube; and plasma poor in growth factors (PPGF), which appeared at the top of the tube. Then
1 mL of the PPGF from each 5-mL tube was discarded. The remaining plasma was collected and centrifuged at 1,500 rpm for 10 minutes. Next 50 mL of 10% calcium chloride and 1,250 units of thrombin were added to the DBP mixture and PRGF to activate platelets in the PRP preparation. After 10 to 15 minutes, a PRGF gel was formed. Machine counts of peripheral blood platelets yielded a mean value of 443,000/mm$^3$, with a range of 400,000 to 505,000/mm$^3$. The mean platelet count of PRP was 1,735,000/mm$^3$, with a range of 1,520,000 to 2,005,000/mm$^3$, a measured increase of 392%.

Wound closure was performed in layers using a 3-0 Vicryl suture (Ailee, Seoul, Korea). Bacitracin ointment was applied over the incision postoperatively. Antibiotic therapy was administered 1 hour before surgery and then twice daily for 3 days.

**HISTOLOGIC PROCEDURE**

At 6 and 12 weeks after implantation, the animals were killed by perfusion with formalin fixative through the left ventricle of the heart. A total of 30 implants were retrieved. The implants and surrounding tissues were immediately washed in saline solution and then immediately fixed in 70% alcohol for at least 72 hours. The specimens were dehydrated in an ascending series of alcohol rinses and embedded using a process that produces thin ground sections with the glycol-metacrylate resin (Spurr Low-Viscosity Embedding Media; Polysciences, Warrington, PA). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disc (low-speed diamond wheel saw 650; SBT, San Clemente, CA) at approximately 200 μm in thickness and ground down to approximately 30 μm in thickness using a lapping and polishing machine (OMNILAP 2000; SBT).

**HISTOMORPHOMETRY**

Three slides were created for each implant. The slides were stained with bone stain (Villanueva osteochrome bone stain; SBT) according to the manufacturer’s instructions. The slides were observed in normal transmitted light under a light microscope (Olympus BX50, Tokyo, Japan). The histomorphometry was performed with a Microvid system (Leitz, Wetzlar, Germany) connected to an IBM personal computer (Armonk, NY).

**STATISTICAL ANALYSIS**

The data were analyzed using the Kruskal-Wallis test in the Statistical Package for the Social Sciences (SPSS) for Windows, version 7.5 (SPSS, Seoul, Korea). Analysis of variance (ANOVA) with a multiple-comparison test was used for intergroup comparison among the groups. The time periods in each group were compared with use of the Wilcoxon rank test. Values of $P < .05$ were considered statistically significant.

**Results**

**CONTROL GROUP (GROUP 1)**

At 6 weeks, there were immature bone trabeculae growing around the implant. There was very little direct contact between the bone and the implant in the medullary and transcortical portions of the implant. However, in the exposed threaded regions, fibrous connective tissue between the bone and the implant was found (Figs 1A, B).

At 12 weeks, the coronal part of the partially regenerated control defects contained connective tissue. Direct contact with new woven lamellar bone was clearly found in the upper and lower transcortical portions of the implant and rarely in the medullary portion (Fig 2A). In the direct contact region, small bone trabeculae partially surrounded the implant surface (Fig 2B).

**GRAFTING WITH DBP (GROUP 2)**

At 6 weeks, it was difficult to distinguish between the traces of DBP and the samples. Fibrous connective tissue in the threaded portions was hardly detected. The formation of new bone in the upper and lower cortical bone and in the marrow cavity was independent of bone propagation in the cortical bone. New bone was formed largely at the implant interface in the upper and lower transcortical portions of the implant, as well as in the medullary portion (Figs 3A, B).

At 12 weeks, new spicules seemed to be more mature than at 6 weeks. The contacts between the new bone and the implants were found largely at the implant surface. Many small bone trabeculae almost completely surrounded two thirds of the implant surface (Fig 4A). The resultant bone formation and its maturity, and the intensity of the marrow between the mature bone, were remarkably increased compared with those at 6 weeks. Histology showed the mature spicules to be woven lamellar bone (Fig 4B).

**GRAFTING WITH DBP AND PRP (GROUP 3)**

At 6 weeks, equal formation of new bone was seen in a wide region around the implant in the transcortical and medullary portions. Compared with group 2, group 3 also showed equal bone formation in the marrow cavity region, with such enhanced bone maturity that it was difficult to differentiate the implant from its surface (Figs 5A, B).

At 12 weeks, the resultant bone formation and its maturity, and the intensity of the marrow between
the mature bone, were increased compared with those at 6 weeks. New bone trabeculae almost completely surrounded the entire implant surface (Fig 6A). Bone trabeculae showed well-formed concentric lamella and haversian systems. The amount of lamellar bone and its maturity in the entire implant surface were more enhanced than those in group 2 (Fig 6B).

**HISTOMORPHOMETRIC ANALYSIS**

The mean percentages of direct implant-bone contact in groups 1, 2, and 3 are shown in Table 1. There was a statistically significant ($P < .05$) difference in new bone formation between the 2 experimental groups and the control group. In addition, there was a significant ($P < .05$) difference between the 2 interexperimental groups.

The mean bone regions in direct contact with the implant threads in groups 1, 2, and 3 are shown in Table 2. There was a statistically significant ($P < .05$) difference in direct implant-bone contact between the 2 experimental groups and the control group in each period. In addition, when comparing the 2 experimental groups,
a significant \((P < .05)\) difference was detected at 12 weeks.

In summary, the following results are obtained: 1) the highest rate of bone ingrowth occurred in group 3, followed by group 2 and then group 1 (control); 2) the contact order of fibrous connective tissue was group 1 (control) \(>\) group 2 \(>\) group 3; 3) the order of bone maturity was group 3 \(>\) group 2 \(>\) group 1 (control); and 4) new bone almost completely surrounded the entire implant surface only in group 3.

**Discussion**

The following are the criteria for ideal graft materials: the ability to facilitate osteogenesis, stability of the implant when placed with the graft, low risk of infection, ease of availability, low antigenicity, and high level of reliability.\(^{23,24}\)

The chosen implant material must provide the proper viable bone to stabilize the implant and facilitate osseointegration. The viability of the implanted
bone is important in the long-term maintenance of the implant.

The success rate of implantation can be increased by guiding the osseointegration of the bone defect with bone grafting on top of the implant. Materials such as autogenous, allogenic, and xenogenic bones, as well as synthetic materials, can be used. The autogenous bone graft has proved to provide a scaffold for bone regeneration. However, its use presents some potential problems, such as the need for a second operative site, resultant patient morbidity, and the possibility of not being able to obtain a sufficient amount of material. 22

Urist3,4 implanted cortical bovine-derived bone matrix that had been freshly prepared and demineralized in 0.6N HCl into ectopic athymic mice and rats. The results of these experiments resulted in ectopic bone formation in the muscle of the mice and rats. These initial studies created widespread interest in the fields of medicine and dentistry. Freeze-dried bone allografts have been used clinically in orthopedic therapy since 1950 because available autogenous bone from

FIGURE 5. Implants grafted with DBP and PRP at 6 weeks. A, Equal formation of new bone was found in a wide region around the implant (Villanueva bone stain, original magnification x10). B, New bone trabeculae (arrows) were in close contact with the implant surface (Villanueva bone stain, original magnification x40).

FIGURE 6. Implants grafted with DBP and PRP at 12 weeks. A, New bone trabeculae almost completely surrounded the entire implant surface (Villanueva bone stain, original magnification x10). B, Bone trabeculae (arrows) showed well-formed concentric lamella and haversian systems (Villanueva bone stain, original magnification x40).
the jaws may be limited. DBP has been the most commonly used bone allograft for more than 20 years. Mellonig reported that DBP can be easily used in the clinical setting and that it has good osteogenic potency. BMP acts on undifferentiated mesenchymal cells to form endochondral bone within 21 days after implantation. BMP induces mesenchymal cell recruitment, differentiation of chondroblasts, cartilage formation, vascular ingrowth, and, ultimately, bone formation. The amount of bone formation is related to the age of the donor and the quantity of BMP implanted. The volume of bone formed is related to the quantity of BMP present. Factors/proteins present in DBP stimulate migration and attachment of cells at the healing site, proliferation of cells, biosynthetic activity by cells, and chondroblastic and osteoblastic cell differentiation.

DBP has been extensively used, often with controversial results. Recent studies showed that osteoinductive proteins, such as BMPs, enhanced osteoblast differentiation but not cell proliferation. In contrast, other researchers reported that DBP has a lower osteogenic capacity and has produced a significantly diminished degree of osseointegration. Becker et al reported that DBP promoted the least amount of new bone within the osseous defects. Pansegrau et al reported diminished integration of implants grafted with DBP. In contrast, Landsberg et al reported that DBP is capable of promoting bone formation around dental implants if complete flap coverage and membrane presence can be maintained throughout the healing phase.

The goal of this study was not to quantify the amount of regeneration that occurred but rather to determine whether any regeneration ever occurred. In the present study, the DBP and PRP grafted defects had a significantly higher percentage of bone.

PRP is an autologous source of platelet-derived growth factor (PDGF) and transforming growth factor (TGF). It is obtained by sequestering and concentrating platelets using gradient density centrifugation.

Blood platelets are a rich source of growth factors, including PDGF, platelet-derived endothelial cell growth factor, and TGF-β. PDGF stimulates the growth of mesenchymal cells such as fibroblasts and vascular smooth muscle cells, whereas platelet-derived endothelial cell growth factor is a mitogen for vascular endothelial cells. TGF-β is a bifunctional regulator of cellular growth, but it also acts as a potent inhibitor for most cell types. TGF-β is a growth and differentiation factor that can be released from many cell types. Because PDGF and TGF-β assist in soft tissue healing and bone mineralization, they can be mixed with the grafting materials into the bone graft site and/or applied to the top layer of the graft. PDGF mixed with autologous bone grafts can accelerate mineralization by as much as 40% during the first year.

Becker et al compared bone promotion around implants that were augmented with expanded polytetrafluoroethylene (ePTFE) barrier membranes alone or in combination with cortical DBP or in combination with PDGF-BB and insulin-like growth factor I (PDGF/IGF-I) in dogs. At 18 weeks, the results showed that, clinically, ePTFE membranes alone and ePTFE membranes with PDGF/IGF-I were equally effective in promoting bone growth around the implants.

In the present study, nongrafted defects showed bone regeneration only in their lower portion. Note that in all control implants, as well as in the inferior half of the experimental implants placed in the host bone, there was no space between the implant surface and the osteocytes of the transplanted DBP, with or without PRP. The histomorphometric results showed a significantly higher percentage of bone-implant contact. This study confirms the portion of the experimental implants placed in the host bone, osseointegrated, and there was a large amount of bone contact achieved with the implant.

In this study, the best new bone formation and bone contract were obtained with DBP and PRP. However, this study has 2 potential limitations. First, different results might be obtained in a clinical setting, because the study used bone from the iliac crest.
where the possibility of bacterial contamination is relatively small compared with clinical harvest sites. Second, this study covered a limited time. Unless there is a specific reason, however, we expect that once bone induction starts, new bone formation will subsequently increase. Comparing the mean percentages of new bone region and bone contact rates of implant interface (Tables 1 and 2), the experimental groups show a statistically significant degree of bone maturation compared with the control group, and when DBP or DBP/PRP was added, the period of implant-bone interface healing decreased. This may have implications in clinical cases where bone defects are managed at the time of implant placement.

References