Use of Particulate Dentin–Plaster of Paris Combination with/without Platelet-Rich Plasma in the Treatment of Bone Defects Around Implants

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Purpose: *To evaluate the effect of particulate dentin-plaster of Paris with and without platelet-rich plasma (PRP) on bone healing and new bone formation around titanium dental implants in a canine model. Histologic sections and histomorphometric analysis of the defects were obtained at 6 and 12 weeks after surgery.* ***Materials and Methods:*** *Three circular bone defects were surgically prepared in iliac crest sites in each of 10 animals. A total of 30 Avana dental implants were placed in the animals. They were self-tapping, screw-type implants, 10 mm in length and 4 mm in diameter, all made of commercially pure titanium. A titanium implant was 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 particulate dentin-plaster of Paris; (3) grafting with particulate dentin-plaster of Paris and PRP.* ***Results:*** *Histologic analysis showed that all of the bone defects surrounding the implants that were treated with particulate dentin-plaster of Paris, with and without PRP, were filled with new bone. The defects that were not treated (control) demonstrated new bone formation only in the inferior threaded portion of the implants.* ***Discussion:*** *Histomorphometric results revealed a higher percentage of bone contact with particulate dentin-plaster of Paris and PRP compared to the control and particulate dentin-plaster of Paris.* ***Conclusions:*** *These results suggested that bone defects around titanium implants can be treated successfully with particulate dentin-plaster of Paris, and that the outcome can be improved if PRP is also used.* (Int J Oral Maxillofac Implants 2002;17:86–94)

Key words: *implants, particulate dentin-plaster of Paris, platelet-rich plasma (PRP)*

A prerequisite for placing dental implants is that a sufficient amount of bone must be available to fully cover the implant and for the implant to support a fixed prosthetic restoration.[1] Bone defects around dental implants are frequently observed when implants are placed in areas with inadequate bone, ie, dehiscence defects, fenestration defects, residual intraosseous defects, defects associated with the extraction sockets, or in situations when the implants are going to fail.[2] In all of these situations, bone regeneration of the defect would improve the long-term prognosis of the implant.[2]

When autogenous bone is harvested from the maxillofacial region, the success rates for augmentation are high.[3–7] However, its application is limited because of some disadvantages, including the need for a donor site, pain, swelling, bleeding, and resorption after grafting. As a result, efforts to develop a substitute material for autogenous bone implantation have been actively carried out.

An ideal bone substitute material should incorporate into the host bone as well as autogenous bone, but unlike autogenous bone, be available in unlimited quantities, either synthetically manufactured or produced naturally.[7,8] Research on allograft and heterograft have also been conducted and

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applied several times to determine suitability for use in clinical settings.[2] However, these studies have faced various problems, including revascularization, immune rejection, and possible disease contamination. It is primarily for this reason that alloplastic materials have been developed. Hydroxyapatite (HA) ceramic materials are the most frequently used alloplastic materials. The use of osteoconductive bone substitutes is controversial, since their use can lead to prolonged healing time, inhomogenous ossification, foreign body reaction, migration of particles, and low bone-implant contact.[3]

Research on grafting materials has been conducted since HA materials were developed.[9,10] It has proven to be difficult to maintain stable fusion between the powder-type HA and the surrounding bone because of its fluid nature. Although many possible different ways to solve this fluidity problem have been introduced, satisfactory results have yet to be attained. Therefore, the authors attempted to determine a way to improve the stability of the materials by using additives. Dental porcelain,[11] dental cement,[12] and plaster of Paris[10,13,14] are additives that have been tried previously. Plaster of Paris was used in this study.

Since Dreesman first attempted the use of plaster of Paris (calcium sulfate), tooth ash and plaster of Paris have been studied as bone substitutes in experimental and clinical studies.[9] Particulate dentin (tooth ash, tooth particles) is readily available from teeth and composed mainly of HA.[15] Plaster of Paris is readily available, easily sterilized, inexpensive, completely and rapidly resorbable, and biocompatible, and has been shown to be well tolerated by tissues. In addition, plaster of Paris is osteoconductive. It is not osteogenic in itself, but in the presence of bone and/or periosteum it almost always becomes osteogenic.[9,16–18] The osteogenic process, referred to as phase I bone, is related to the number of cells transplanted and dictates the amount of new bone that will form beyond the original dimension. It is directly proportional to the density of cells transplanted.[19]

Methods to strengthen the power of osteogenesis and osteoinduction are being studied. In this study, the latest osteogenesis technique using platelet-rich plasma (PRP) was employed to facilitate the osteogenesis or osteoinduction of plaster of Paris, rather than its osteoconduction.

To the authors' knowledge, the osteogenic capability of particulate dentin-plaster of Paris, with and without PRP, to fill the bone defects around titanium dental implants has not been previously reported. The purpose of this study was to evaluate the effect of particulate dentin-plaster of Paris with and without platelet-rich plasma (PRP) on bone healing and new bone formation around titanium dental implants in a canine model.

MATERIALS AND METHODS

Materials Studied

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. Preoperatively, the dogs were anesthetized with an intramuscular injection of ketamine HC1 (Ketalar, Yuhan, 10 mg/kg) and xylazine (Rompun, Bayer, 3 mg/kg). During anesthesia induction, each dog received cefazoline (22 mg/kg, IV). To aid hemostasis in the areas of the planned soft tissue incisions and dissection, 2% lidocaine HC1 with 1:100,000 epinephrine was injected in the ilium.

The surgical procedure was initiated by an incision, and mucoperiosteal flaps were gently raised. Three circular bone defects 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, Busan, Korea) were used as the experimental implants. The implants were self-tapping, screw-type implants 10 mm in length and 4 mm in diameter, all made of commercially pure titanium. A titanium implant was then placed centrally in each defect 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. In each dog, the defects were treated with 1 of the following 3 treatment modalities: *(1)* no treatment (control); *(2)* grafting with particulate dentin-plaster of Paris; *(3)* grafting with particulate dentin-plaster of Paris and PRP.

All of the implants were submerged with tension-free mucoperiosteal flaps using the vertical mattress suturing technique.

Particulate Dentin Preparation Procedure

Particulate dentin was produced using the following method. First, removal of the foreign matter and soft tissue attached to the teeth's surface was achieved by soaking the relatively good teeth in hydrogen peroxide. Next, the teeth were ground as completely as possible using a mortar and pestle after heating them in a furnace at a high temperature (2,192°F) for 2 hours. The teeth were then filtered using a mesh tray (Sieve No. 100), and the filtered powder was ground minutely 2 or 3 times. The final particle size of the

particulate dentin was 0.149 mm. Disinfection was carried out in an autoclave after placing the powder into a beaker filled with distilled water. After disinfection, the tap water and floaters were carefully removed using a pipette, and the distilled water and residue were placed back into the beaker, which was shaken. Sterilization was done after storing the solution for 1 day. This autoclaving process was repeated 5 times to completely remove any foreign matter. After the final sterilization, the distilled water and residue were removed and dried using a drying oven. After autoclaving, the yielded powder was used as implant material. The prepared materials were stored, using ethylene oxide after disinfecting them. Measurements of their weight were made for convenient use.

Particulate Dentin-Plaster of Paris Preparation Procedure

The particulate dentin and plaster of Paris were mixed using saline at a ratio of 2:1 by weight and placed into the defect. Once it had dried, the material was sculpted with a bur so as to match the contour of the remaining bone.

Platelet-Rich Plasma Preparation Procedure

Blood was obtained several minutes prior to the administration of anesthesia. Ten ml of blood were drawn from each dog using 5 ml tubes, which 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. One ml of the PPGF from each 5 ml tube was discarded. The 6 remaining plasma was collected and centrifuged at 1,500 rpm for 10 minutes. Fifty ml of 10% calcium chloride and 1,250 units of thrombin were then added to the particulate dentin/plaster of Paris mixture and PRGF to activate platelets in the PRP preparation. After 10 to 15 minutes, a PRGF gel was formed.

Histologic Procedure

At 6 and 12 weeks after implantation, the animals were sacrificed 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 at 4°C for 6 days. The specimens were dehydrated in an ascending series of alcohol rinses and embedded using a process which produces thin ground sections with glycol-methacrylate 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, and ground down to approximately 30 µm using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, CA).

Histomorphometry

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

Statistics

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

RESULTS

During the test periods, the experimental animals remained in good health. At sacrifice, no clinical signs of inflammation or adverse tissue reactions could be seen. All implants were still in situ at sacrifice.

On histologic examination, apparent osseointegration of both experimental groups was found. There was no space found between the implant surface and new bone of the transplanted particulate dentin-plaster of Paris with or without PRP.

Group 1. At 6 weeks, there were immature bone spicules growing around the periosteum and endosteum near the marrow cavity. Direct contact between the bone and the implant was clearly seen in the lower portion of the cortical bone in the periosteum and the endosteum. However, in the exposed threaded areas, small amounts of fibrous connective tissue between the bone and the implant were found (Fig 1).

At 12 weeks, the coronal part of the partially regenerated control defects did not contain connective tissues. Direct contact of new lamellar bone was found in the coronal part, as well as in the medullary compartment of the implant (Fig 2).

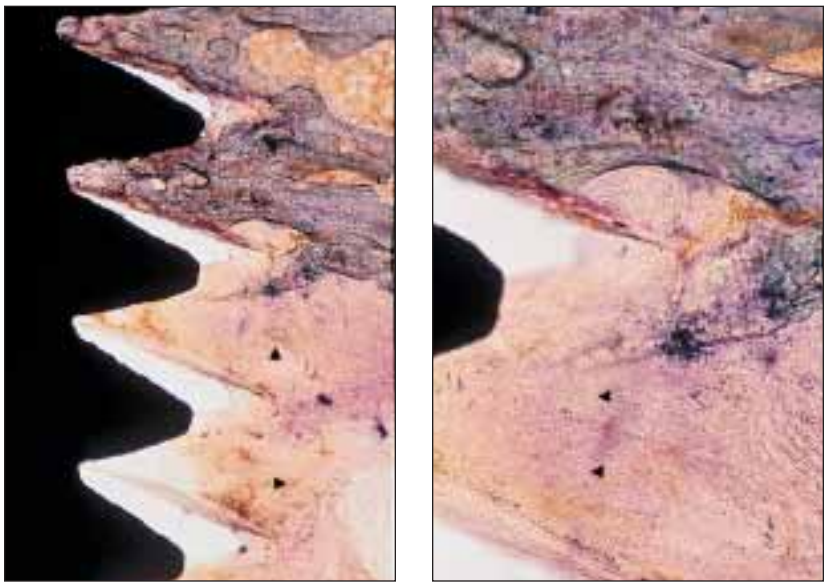

Fig 1 At 6 weeks; control. There were immature bone trabeculae *(arrows)* in the lower portion of the cortical bone. Fibrous connective tissue *(arrowheads)* between the bone and the implant were also observed (Villanueva bone stain; original magnification, left ×40; right ×100).

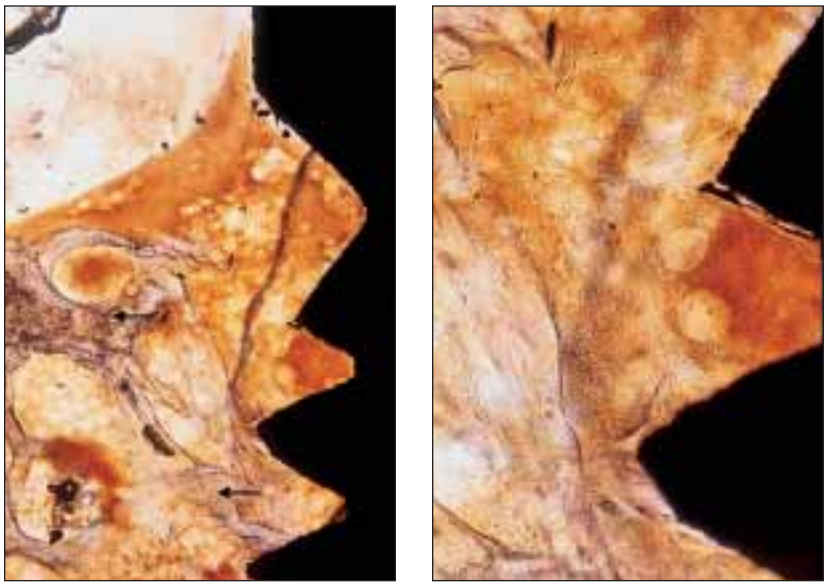

Fig 2 At 12 weeks; control. New bone trabeculae of woven-lamellar type *(arrows)* were seen in the coronal part of the partially regenerated control defects (Villanueva bone stain; original magnification, left ×40; right ×100).

Group 2. At 6 weeks, the particulate dentin-plaster of Paris particles were incorporated into the newly formed bone. Active bone formation was found in the periosteum and marrow cavity of the cortical bone and the endosteum, when compared to the control at 6 weeks. This formation reached the coronal portion of the implant. Thus, there was almost no fibrous connective tissue contact found in the threaded portion (Fig 3). In the upper and lower portions of the periosteum and endosteum areas, newly created bone had formed largely around the implant interface. However, in the marrow cavity area, bone was in contact with the implant only by virtue of the limited formation in the threaded portion.

At 12 weeks, new bone spicules were found to be more mature compared to those at 6 weeks, and contact between the new bone and the implant was observed. The new bone, apart from the implant interface near the marrow cavity, was trabecular. However, the bone in the contact area between the original bone and the implant was found to be mature lamellar bone (Fig 4).

Group 3. At 6 weeks, identical formation of new bone spicules was found in the periosteum and marrow cavity of the cortical bone and in the endosteum near the implant. The particulate dentin-plaster of Paris particles were also incorporated into the newly formed bone. Fibrous connective tissue contacts were found only in the interface of the upper portion of the periosteum. In comparison to the group 1 (control) and group 2, the new bone in this group was even found in the marrow cavity area with bone

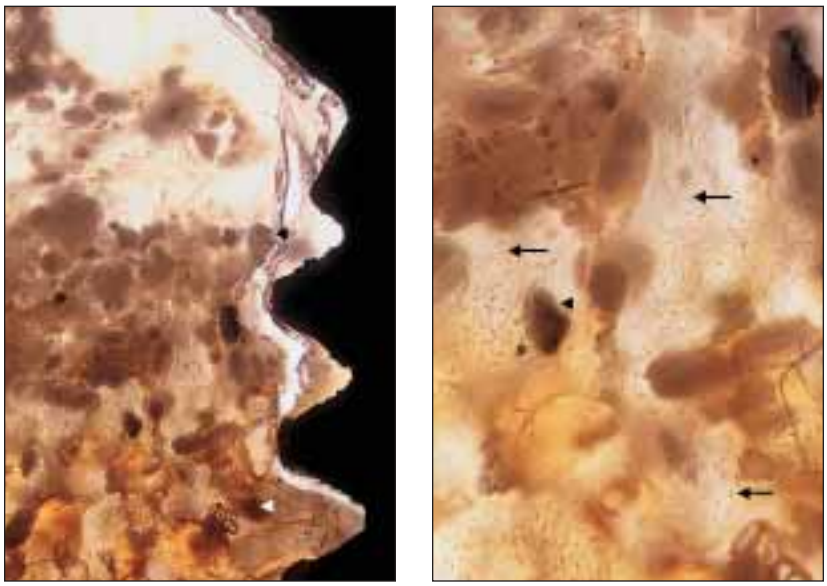

Fig 3 At 6 weeks; group 2. New bone of woven type *(arrows)* were observed in the threaded portion of the implant. Particulate dentin and plaster of Paris particles *(arrowheads)* were also incorporated into the newly formed bone (Villanueva bone stain; original magnification, left ×40; right ×100).

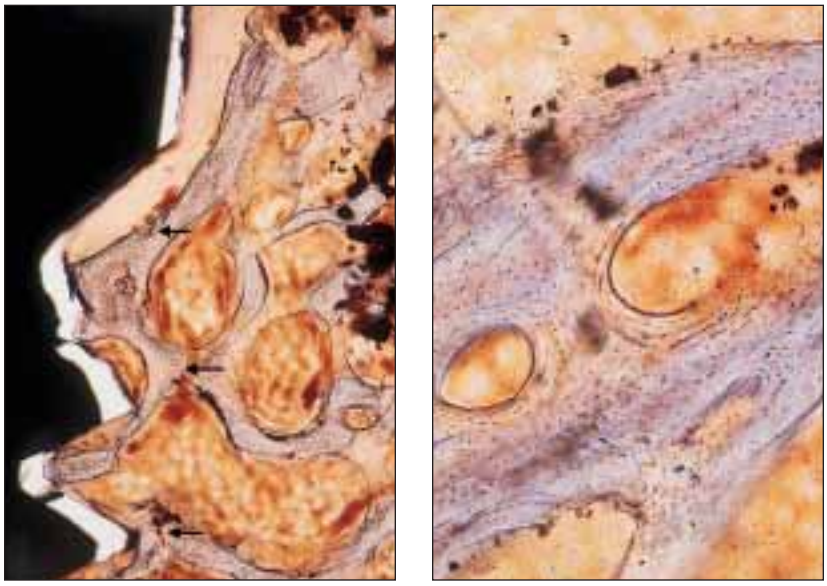

Fig 4 At 12 weeks; group 2. New trabecular bone of lamellar type *(arrows)* were seen in the threaded portion of the implant. There were almost no fibrous connective tissue contact found in the threaded portion (Villanueva bone stain; original magnification, left ×40; right ×100).

maturity, making it difficult to distinguish the newly formed bone from the original bone at the entire implant surface (Fig 5).

At 12 weeks, the majority of particulate dentin-plaster of Paris particles were incorporated into the newly formed bone in intimate contact with the implant. The resulting bone formation and its maturity, and the marrow space between the mature bones, were remarkably reduced compared to that seen at the 6-week mark. Mature bone spicules were shown to have lamellar bone with various layers composed of different patterns of bone cells, and its equivalent maturity was found largely in new bones (Fig 6). In some samples, particulate dentin and plaster of Paris were found, and bone regeneration had occurred near these areas.

Histomorphometric Analysis

The mean percentages of new bone formation in groups 1, 2, and 3 are shown in Table 1. There was a statistically significant difference in new bone formation between the 2 experimental groups and the control group in each period ($P < .05$). In addition, when comparing the 2 interexperimental groups, significant difference was detected in the 6-week groups. There was a statistically significant difference between the 6-week group and the 12-week group in group 2.

The mean bone areas 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

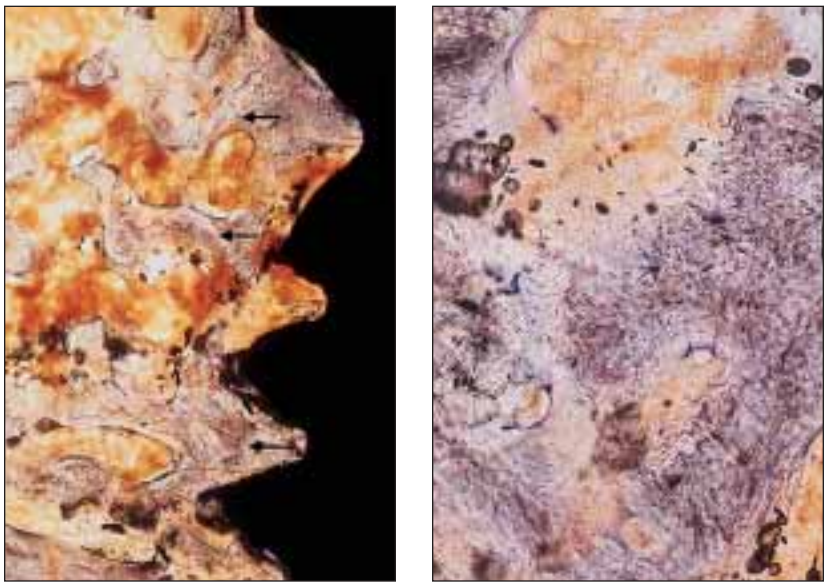

Fig 5 At 6 weeks; group 3. New trabecular bone of lamellar type *(arrows)* almost completely surrounded the implant surface (Villanueva bone stain; original magnification, left ×40; right ×100).

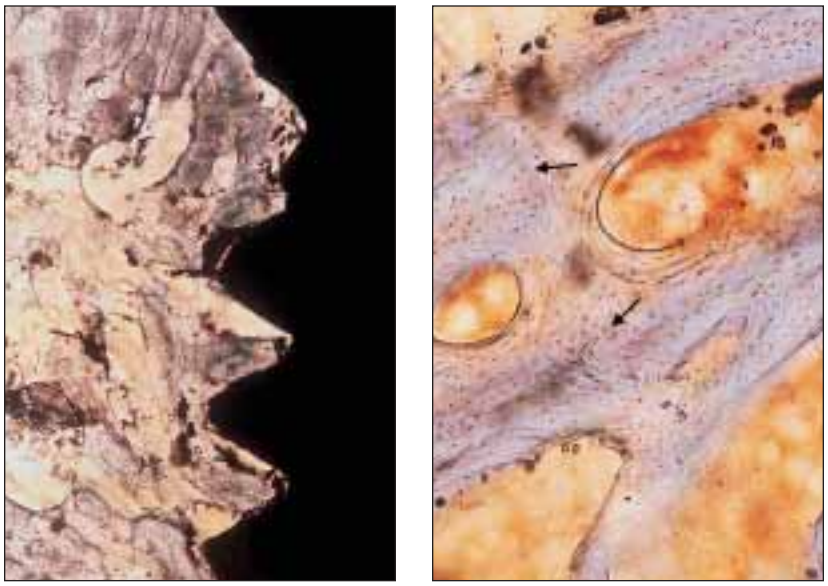

Fig 6 At 12 weeks; group 3. Newly formed bone characteristically has shown lacunae *(arrows)* containing osteocytes arranged in lamellae (Villanueva bone stain; original magnification, left ×40; right ×100).

each period. In addition, when comparing the 2 experimental groups, a significant ($P < .05$) difference was detected at 6 weeks.

In summary, the following findings were determined:

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).

DISCUSSION

The threaded portion of all Avana dental implants in the present study was osseointegrated. The results demonstrated that surgically created bone defects, similar to those observed around failed dental implants, healed with complete bone fill and osseointegration to the implant surface when grafted with a particulate dentin-plaster of Paris, whether or not the defect was grafted with PRP.

In attempts to stimulate osteogenesis and reconstruct bone defects in cases of various injuries, diseases and congenital malformation in dentistry, orthopedics, and neurosurgery, various types of bone grafts or biomaterials have been used to treat the defects.[2,20]

Table 1 Mean Percentages of Direct Implant-Bone Contact in Groups 1, 2, and 3, at 6 and 12 Weeks After Placement

	Mean ± SD		
Time period	Group 1	Group 2	Group 3
6 weeks	24 ± 18.4	43 ± 15.4*	72 ± 16.4*,†
12 weeks	30 ± 6.7	60 ± 18.0*	57 ± 7.6*

*Statistically significant difference relative to Group 1.
†Statistically significant difference relative to Group 2.
SD = standard deviation.

Table 2 Mean Bone Area in Implant Threads of Direct Implant-Bone Contact in Groups 1, 2, and 3, at 6 and 12 Weeks After Placement

	Mean ± SD		
Time period	Group 1	Group 2	Group 3
6 weeks	16 ± 11.0	47 ± 24.1*	75 ± 8.2*,†
12 weeks	25 ± 18.0	58 ± 28.5*	78 ± 2.9*

*Statistically significant difference relative to Group 1.
†Statistically significant difference relative to Group 2.
SD = standard deviation.

Since HA materials were developed, research on grafting materials has been steadily carried out. HA is the primary inorganic, natural component of bone. It is extremely biocompatible and bonds readily to adjacent hard and soft tissues.[21] However, its high cost and its complicated application to surgery are shortcomings that have been identified. One major challenge is that it can create significant problems, particularly with rejection and the inability of the grafting material to develop a stable fusion with the surrounding bone because its fluid nature facilitates changes in shape.

Plaster of Paris made of calcium sulfate hemihydrate seems to be promising because of its long history of safe use and characteristic complete resorption followed by bone formation.[22] Plaster of Paris is easy to use and disinfect during surgery. It is also available at a reasonable price and can even hinder the fluidity of the implant. Its average solidity enables it to resist rupture after consolidation. Although plaster of Paris can hardly guide and induce new bone formation by itself, its resorptive characteristics and biocompatibility can assist in acting as a bonding agent when mixed with other materials. In addition, the resorption rate becomes faster and invasion around the tissue is facilitated as the material's density decreases.

The greatest advantages of plaster of Paris are its ability to bond firmly with adjacent bone, and its ability to guide new bone formation which occurs in association with resorption of the plaster.[23] Plaster of Paris has been used in the treatment of bone defects in the fields of orthopedics, otorhinolaryngology, and oral and maxillofacial surgery. De Leonardis and Pecora[24] reported that the overall success rate for 130 placed implants at 1 year postimplantation was 98.5%. In that study, a sinus augmentation procedure was performed using calcium sulfate as the sinus grafting material. This also indicated that calcium sulfate could be a suitable material for sinus augmentation.

Najjar and coworkers[10] attempted to determine whether the addition of calcium sulfate to HA implant material would improve its working properties without adversely affecting its osseointegration capability. No sign of extensive chronic inflammatory response was detected. The highest rate of bone ingrowth occurred with an HA composite (HA plus calcium sulfate), followed by HA alone. Bone was deposited directly on the surface of HA and HA composite implant materials. Calhoun and associates[17] noted that natural gypsum hardly showed any tissue reactions, such as inflammation. McKee and Bailey[25] observed that the worst problem resulting from the use of plaster of Paris was infection. They also reported that because such infection can be controlled effectively, successful replacement of the calcium sulfate for new bone formation can occur either with or without the presence of the periosteum.

Kim and coworkers[13] microscopically examined tooth ash and plaster of Paris. Implanted particles were divided into small particles and the number of particles decreased gradually over the first 8 postoperative weeks. Most of the implanted sites were repaired by newly formed bone by the 12th postoperative week.

Clinical results have shown that inflammation is a reaction manifested in the early healing stage. However, in the follow-up study, it was determined that inflammation and foreign body reactions could be resolved successfully without any major complications. Therefore, it was concluded that the particulate dentin-plaster of Paris mixture is a useful and readily available material as a bone substitute in the maxillofacial area.[15]

To enhance bone formation, 2 approaches were considered. First, during the preparation of the particulate dentin, hydrogen peroxide was used in the cleaning procedure. Since this material is known to destroy the activity of BMP and transforming growth factor–β (TGF–β) in the dentin matrix, this

method was not used. Second, particulate dentin and plaster were sterilized and stored in ethylene oxide gas, which is very toxic. Unfortunately, it cannot be assumed that all of the ethylene oxide was removed from the powder before use. This process probably could affect the results.

PRP gel, an autologous formulation of fibrin glue, is the latest osteogenesis technique for strengthening the effect of bone graft substitutes.[26] PRP is obtained by sequestering and concentrating platelets using gradient density centrifugation. This technique produces a concentration of human platelets of 338%.[27] PRP gel has been used successfully in the area of oral and maxillofacial surgery in conjunction with ablative surgery of the maxillofacial region, mandibular reconstruction, in bone grafting after sinus lift, root apex removal, periapical cyst removal, tooth extraction, in alveolar ridge augmentation, surgical repair of alveolar clefts and associated oro-antral/oro-nasal fistulae, and in adjunctive procedures related to the placement of osseointegrated implants.[26,28] In summation, the use of PRP with allogenic or xenogenic bone facilitates new bone formation when autogenous bone grafting is difficult or not indicated for use.

PRP, which is a comprehensive term, can be divided into PRP and platelet concentrate (PC). PC is practically used, while PRP refers to the mixed plasma and buffy layer among the blood components. When PRP, which has much larger platelets than normal ($150 \approx 400 \times 10^3$/dl), is mixed with $CaCl_2$ and thrombin and added to the bone graft material, a favorable prognosis can be expected. PC influences the bone healing process because it secretes platelet-derived growth factor (PDGF), TGF–β, and other growth factors.[19,27,29]

Tayapongsak and associates[30] used autologous fibrin adhesive (AFA) in a series of 33 cases. Bony incorporation and remodeling were detected radiographically at the fourth postoperative week compared to the eighth week for bone grafts without AFA. According to their results, in addition to adhesive and hemostatic properties, AFA helped the remodeling process begin in approximately half the time by providing the substratum for the migration of mesenchymal cells, accelerating the revascularization and migration of fibroblasts, stimulating the growth of both fibroblasts and osteoblasts, and slowing down the multiplication of microorganisms.

Marx and coworkers[27] reported that adding PRP to grafts resulted in a radiographic maturation rate of 1.62 to 2.16 times that of grafts without PRP. On histomorphometric analysis, there was also greater bone density in grafts to which PRP had been added (74.0% ± 11%) than in grafts without PRP (55.1% ± 8%).

In the present study, nongrafted defects demonstrated bone regeneration in their lower portions only. The coronal portions of the partially regenerated control defects contained connective tissue. No dehiscences were observed in the experimental groups of defects. All of the bone defects grafted with particulate dentin-plaster of Paris material showed complete healing with bone-fill. The newly formed bone extended coronally so that the upper surface of the implant was covered. Direct contact between the bone and implant was frequently established. The bony defects filled with particulate dentin-plaster of Paris and PRP material showed complete healing. The majority of particulate dentin-plaster of Paris particles were incorporated into the newly formed bone, with an intimate contact with the implant. In addition, there was a progressive increase in the new bone volume over time.

CONCLUSION

The histomorphometric results showed significantly higher percentages of bone-implant contact in the grafted sections. This study confirmed that the portion of the experimental implants placed in the host bone osseointegrated and a large amount of bone contact was achieved with the implant. Results of the present study suggest the efficacy of particulate dentin-plaster of Paris in the treatment of bone defects around dental implants. Because of the high rate of new bone formation with no major observed side effects, it was concluded that particulate dentin-plaster of Paris with PRP has the potential to become a practical treatment method for bone defects around dental implants.

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