Influence of Platelet-rich Plasma Added to Xenogeneic Bone Grafts on Bone Mineral Density Associated with Dental Implants

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Purpose: *The purpose of this study was to assess the bone mineral density changes after bone regeneration therapy using xenogeneic demineralized freeze-dried bone graft (DFDBG) plus platelet-rich plasma (PRP) and DFDBG alone in 3-wall peri-implant defects in dogs.* ***Materials and Methods:*** *The mandibular premolars and molars of 9 adult hound dogs were removed surgically, and 90 sites were prepared for implant placement. Before implant placement, a total of 162 mesial and distal 3-wall peri-implant defects were surgically created. Defects were randomly assigned to three groups: DFDBG + PRP, DFDBG alone, and no treatment. Animals were sacrificed at 1, 2, and 3 months, and specimens were subjected to bone mineral density (BMD) and bone mineral content (BMC) analysis with a peripheral dual x-ray absorptiometry densitometer.* ***Results:*** *The effect of treatment on BMD and BMC differed significantly by month of sacrifice (*P = *.030 and* P = *.035 for the month-by-treatment interactions, respectively). BMD differed significantly between peri-implant defects treated with DFDBG alone and untreated defects at 3 months (mean BMD of 0.6667 for DFDBG alone versus 0.5606 for untreated defects;* P < *.001). BMC also differed significantly between peri-implant defects treated with DFDBG alone and untreated defects at 3 months (mean BMC of 0.0276 for DFDBG alone versus 0.0236 for untreated defects;* P = *.001). No other pairwise comparison of the treatments within each month of sacrifice or at the overall treatment effect across all three months demonstrated significant differences.* ***Discussion:*** *PRP has been proposed as an autogenous source of growth factors, which may increase the speed and completeness of healing. This study did not demonstrate a significant improvement in BMD or BMC when PRP was combined with DFDBG. Defects where grafting material was used, either with or without PRP, did demonstrate slightly greater BMD and BMC than those left untreated.* ***Conclusion:*** *This study found that the addition of PRP to xenogeneic bone grafts did not significantly alter BMD or graft maturity levels in this animal model.* Int J Oral Maxillofac Implants 2005;20:526–532

Key words: *bone mineral density, dual energy x-ray absorptiometry, growth factors, guided bone regeneration, platelet-rich plasma*

Guided bone regeneration (GBR) has emerged as a treatment in the management of osseous defects associated with dental implants.[1,2] Autologous cancellous bone has been reported to be the ideal grafting material to augment areas of bone deficiency.[3,4] However, when the availability of autologous bone is limited, alternative materials such allografts, xenografts, and alloplastic bone substitutes have been used.[5,6]

Two commonly used allografts are demineralized freeze-dried bone graft (DFDBG) and freeze-dried bone graft (FDBG), but controversy exists with respect to the osteoinductive/osteoconductive potential of these materials. Previous studies have failed to demonstrate conclusive evidence of the superiority of 1 material or the other.[7–10]

Recently, platelet-rich plasma (PRP) has been described as a source of autogenous growth hormones that may improve healing following surgical intervention. PRP is obtained from autologous blood and is

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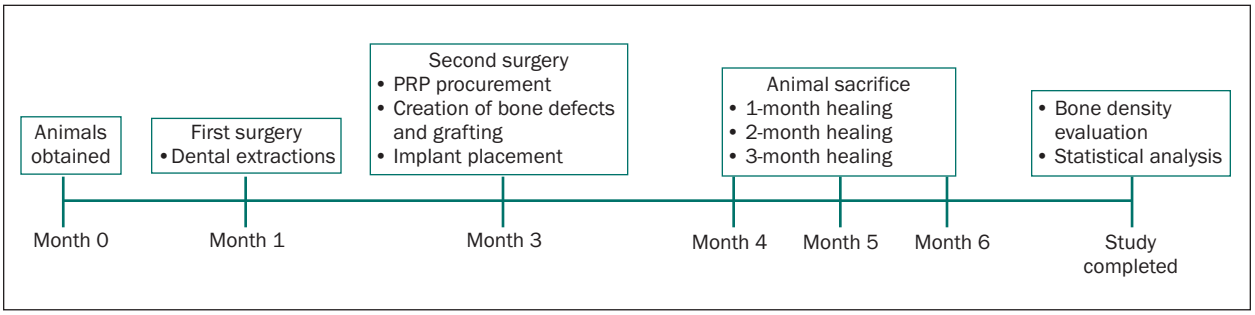

Fig 1 Study timeline.

used to deliver growth factors in high concentrations to the site of the bone defect or a region requiring augmentation.[11,12] Several clinical reports suggest that the addition of PRP to bone grafts enhances bone mineral density (BMD) and graft maturation.[12–16]

The evaluation of bone density changes in the jaw bones in peri-implant regions is of interest when studying the healing response after bone grafting procedures, but accurate evaluation of these factors through noninvasive means has proven difficult.[17–22] Several methods have been suggested for evaluation of bone density changes, including intraoral radiographs, extraoral radiographs, central and peripheral quantitative computer tomography (QCT), magnetic resonance imaging (MRI), single-photon absorptiometry (SPA), histomorphometric analysis, and central and peripheral dual energy x-ray absorptiometry (DEXA).[23–35] Numerous authors have reported the use of DEXA with good success.[18,20,22,36,37]

The purpose of this study was to assess BMD changes through the use of DEXA after bone regeneration therapy using DFDBG plus PRP and DFDBG alone in 3-wall peri-implant defects in dogs.

MATERIALS AND METHODS

The study sample, 9 fully mature (older than 2 years) adult male hound dogs, was divided into 3 equal groups. One group was allowed a healing period of 1 month, another a healing period of 2 months, and the third a healing period of 3 months. The design of this study is depicted in Fig 1. Nine sites on each side of the mandible were studied; namely, the mesial and distal aspects of the 5 implants placed per side in the premolar-molar region. To maintain balance in the treatments, the distal aspect of the most anterior implant was not studied. Three types of treatments (DFDBG + PRP, DFDBG, and no treatment), denoted by A, B, and C, were randomly assigned to the sites in a balanced design. Within each group, each dog was studied using a separate treatment randomization schedule. The defect sites of adjacent mesial and distal aspects of adjacent implants were assigned the same treatment to avoid contamination or carryover effects. The Institutional Animal Care and Use Committee (IACUC) at Mayo Clinic Rochester approved this study.

Surgical extraction of the mandibular right and left premolar and molar teeth was accomplished in the first month of the study. At the time of surgery, each dog was administered 12.5 mg/kg intravenous 4% methohexital (Brebital; John Medical Industry, St Louis, MO). The animals were intubated following induction of general anesthesia and maintained inhaling 1% halothane anesthetic, along with a 50% mixture of nitrous oxide and oxygen. Prior to tooth extraction, an anesthetic agent was infiltrated locally around surgical areas, and 2% lidocaine hydrochloride with 1:100,000 epinephrine (2% Xylocaine Dental; AstraZeneca, York, PA) was used to obtain hemostasis and postoperative analgesia.

Full-thickness mucoperiosteal flaps were reflected to expose underlying bone. The second, third, and fourth premolars and the first molar were then extracted bilaterally as atraumatically as possible using midcoronal facial and lingual sectioning with a high-speed handpiece with a sterile water–cooled bur and standard dental forceps. Flaps were repositioned and held in place by interrupted 4-0 polyglactin 910 sutures (Vicryl; Johnson & Johnson/Ethicon, Somerville, NJ).

Following surgery, the dogs were administered 0.2 to 0.4 mg/kg butorphanol (Torbugesic, Aveco, Fort Dodge, IA) intramuscularly every 2 to 5 hours as needed for postoperative discomfort. Soft food (Science Diet; Hill's Nutrition, Topeka, KS) was given to the animals for the remainder of the study.

After 2 months of healing, and under the same operating conditions described for the dental extractions, the PRP was procured using the SmartPrep Platelet Concentrator Centrifuge System (Harvest Technologies, Plymouth, MA). This system consists of a microprocessor-controlled desktop centrifuge with

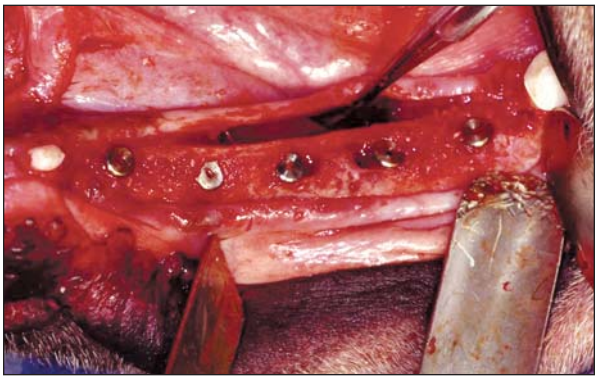

Fig 2 Clinical view of surgically created 3-wall peri-implant defects in the canine mandible.

automatic decanting capacity for PRP and a dual-chambered sterile processing blood container. The system is composed of individually packaged sets of disposable materials that are designed to come into contact with blood. The PRP disposable kit was used for the preparation of 7 to 10 mL of PRP.

The first step in obtaining the PRP was to draw 9 mL of acid citrate dextrose-anticoagulant (ACD-A) into a 60-mL syringe provided with the disposable kit. Of this ACD-A, 2 mL were injected into the plasma chamber of the processing disposable. To process the PRP, 48 mL of venous blood was withdrawn from the dogs 30 minutes before the surgery; this blood was mixed with the 7 mL of ACD remaining in the 60-mL syringe. The syringe was inverted several times to ensure adequate mixing of the blood with the ACD-A to prevent coagulation. Once this had been done, the anticoagulated blood was transferred to the blood chamber of the processing disposable. The start button of the centrifuge was pressed, and after 2 centrifuging cycles, a short- and a long-duration spin, the PRP was separated from the supernatant, or platelet-poor plasma (PPP). The entire process was entirely automatic and was completed in approximately 12 minutes. After centrifugation, approximately two thirds of the PPP were removed using a blunt cannula with a sterile 10-mL syringe. The PRP was then resuspended in the remaining PPP, creating a very concentrated PRP solution.

Subsequently, a midcrestal incision was made and full-thickness flaps, both buccal and lingual, were reflected to expose the alveolar bone. After implant osteotomies were done, a 3-wall bony defect was created surgically in the mesial and distal aspects of the osteotomy sites using a surgical fissure bur in a high-speed air-driven handpiece with sterile saline irrigation. The size of the defect was standardized to approximately 2 mm in a mesiodistal direction, 3 mm in a buccolingual direction, and 5 mm in a coronoapical direction. The dimensions of the defects were measured using a periodontal probe. After that, endosseous titanium implants (Brånemark System; Nobel Biocare, Göteborg, Sweden) were placed in the mandible following a standard implant placement technique. The prepared defects received one of the three treatment modalities according to the randomization schedule. Each defect received *(1)* DFDBG + PRP, *(2)* DFDBG alone, or *(3)* no treatment. No membrane barriers were placed (Fig 2). The bone grafts were reconstituted with sterile saline a few minutes before their application. The PRP was activated to achieve coagulation and release of growth factors by using a special double syringe (LK/2 applicator Harvest Technologies, Plymouth, MA) that equally mixed the PRP and 1 mL of 10% calcium chloride/5,000 units of topical bovine thrombin (Thrombin-JMI; Jones Medical Industries, St Louis, MO) while these solutions were applied to the DFDBG.

After PRP application, the coagulated grafting material was transferred to the defect sites. DFDBG was combined with PRP because it is a radiolucent demineralized carrier that possesses regenerative properties and does not interfere with bone density measurements. The surgical flap was repositioned and closed with 4-0 polyglactin 910 suture (Vicryl). Postsurgical care for these animals was the same as after the dental extractions.

At 1, 2, and 3 months after osseous regenerative therapy, 3 animals, respectively, were euthanized by an injection of an overdose of sodium pentobarbital (Sleepaway Fort Dodge, Overland Park, KS). Following the sacrifice, the mandibles were resected using a band saw (Hobart, Troy, OH) and fixed in formol 10% for 24 to 48 hours.

To investigate if the PRP addition to DFDBG really improves the BMD of the regenerated bone, bone density measurements were accomplished with the use of a DEXA mouse densitometer (PIXImus Research Densitometer; GE Healthcare Lunar, Madison, WI). The system has an ultra-high resolution of 0.18 × 0.18-mm pixels and consists of a peripheral densitometer attached to a Pentium-processor portable laptop computer programmed with the Lunar software and connected to a Ink-Jet Desktop printer (HP 895; Hewlett-Packard, Palo Alto, CA).

To calculate the accuracy of the system, at the beginning of each day of measurements, a quality control plot was generated using a phantom of a known density value (0.0580 g/cm^2, 12.9% fat). The measurable phantom density value on the day of the measurement was used to determine the calibration factor to be used for adjustment of the BMD values obtained that day.

The specimens prepared with ethanol 70% were positioned using a grid provided by the manufacturer of the densitometer. The measurements were performed at the mesial and distal aspects of the treatment site, 1 mm away from the implant surface. The region of interest (ROI) was limited to 4 mm^2. A series of 3 repeated measurements of each region without repositioning the specimens were taken (Fig 3).

After each measurement, the densitometer provided a bone density report, which consisted of mg/cm^2 bone mineral content (BMC) in mg and area of bone in cm^2.

Statistical Analysis

Analysis of variance (ANOVA) models were fit to assess treatment differences in BMD, BMC, and area of bone, respectively. Each ANOVA model included effect terms for duration of healing (1, 2, or 3 months), animal within healing time, side (left or right), treatment (DFDBG + PRP, DFDBG, or no treatment), and the treatment-by-healing time interaction. Contrast statements were used to evaluate the pairwise treatment differences in the presence of a significant treatment-by-healing time interaction effect or overall treatment effect. The ANOVA models assume that the outcome measurements follow a Gaussian (normal) distribution. Natural logarithmic transformations were applied to all of the measurements to satisfy this assumption. All calculated *P* values were 2-sided, and *P* values less than .05 were considered statistically significant. No adjustments were made for multiple comparisons. Statistical analyses were performed using the SAS software package (SAS Institute; Cary, NC).

RESULTS

The measurements from the bone density reports are summarized by healing time and treatment in Table 1. The effect of treatment on BMD differed significantly by month of healing, with increased density being observed as healing time reached the prescribed 3-month maximum ($P = .030$ for the month-by-treatment interaction). This observation was true for both treatment methods and the negative control. Among the animals allowed to heal for 3 months, the mean BMD was significantly higher among peri-implant defects treated with DFDBG alone compared to defects that received no grafts (negative control) (mean BMD, 0.6667 versus 0.5606, $P = .001$). No other pair-wise comparison of the treatments within each healing time (month 1 or month 2) demonstrated a statistically significant difference.

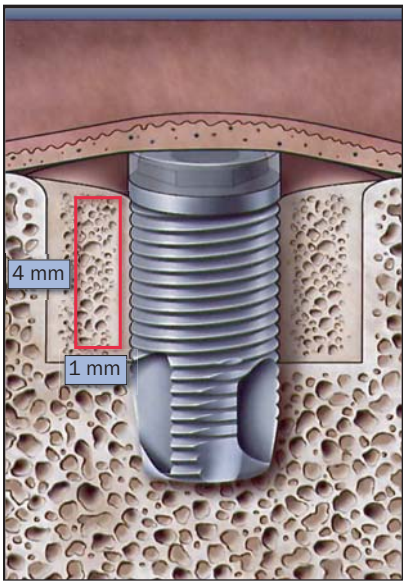

Fig 3 Schematic shows the area subjected to DEXA measurements.

The effect of treatment on BMC also differed significantly by month of healing ($P = .035$ for the month-by-treatment interaction). The only pairwise treatment comparison within each month of healing that was statistically significant occurred between peri-implant defects treated with DFDBA alone and defects that received no grafts (negative control) among animals sacrificed at 3 months (mean BMC of 0.0276 versus 0.0236; $P = .001$). There was no evidence that the effect of treatment on bone area differed by month of healing ($P = .592$ for the month-by-treatment interaction). In addition, there was no evidence of an overall treatment effect across all 3 months ($P = .913$) (Table 2).

DISCUSSION

The properties of PRP are based on the premise that the production and release of multiple growth and differentiation factors contained in platelets could improve healing of surgical defects. It has been reported that these growth factors are stored in the platelet α granules and consist of platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), insulin-like growth factor (IGF), and platelet factor 4 (PF-4).[38]

Marx and associates[12] have proposed a simplified theory of bone healing that focuses on the regenerative potential of 2 growth factors (PDGF and TGF-β). Their theory suggests that PRP delivers a highly concentrated dose of autologous platelets containing a

Table 1 Bone Density Analysis by Healing Time and Treatment

	DFDBG + PRP	DFDBG	No treatment
Month 1			
BMD (mg/cm^2)	0.4894 (0.1102)	0.4674 (0.1096)	0.4808 (0.0883)
BMC (mg)	0.0208 (0.0049)	0.0201 (0.0050)	0.0207 (0.0040)
Bone area (cm^2)	0.0424 (0.0014)	0.0427 (0.0012)	0.0430 (0.0009)
Month 2			
BMD (mg/cm^2)	0.4645 (0.0783)	0.4317 (0.0723)	0.4259 (0.0587)
BMC (mg)	0.0188 (0.0028)	0.0177 (0.0029)	0.0174 (0.0028)
Bone area (cm^2)	0.0404 (0.0023)	0.0409 (0.0020)	0.0406 (0.0023)
Month 3			
BMD (mg/cm^2)	0.6061 (0.0821)	0.6667 (0.0878) *	0.5606 (0.1100) *
BMC (mg)	0.0255 (0.0035)	0.0276 (0.0038) *	0.0236 (0.0045) *
Bone area (cm^2)	0.0421 (0.0017)	0.0415 (0.0023)	0.0422 (0.0018)

*$P < .001$.
Data shown are means (SDs).

Table 2 Bone Density Analysis by Treatment (Grouped Healing Times)

	DFDBG + PRP	DFDBG	No treatment
BMD (mg/cm^2)	0.5190 (0.1082)	0.5209 (0.1372)	0.4904 (0.1035)
BMC (mg)	0.0217 (0.0047)	0.0218 (0.0058)	0.0206 (0.0046)
Bone area (cm^2)	0.0416 (0.0020)	0.0417 (0.0020)	0.0420 (0.0020)

Data shown are means (SDs).

variety of biologic mediators that can be applied directly to the healing site to enhance subsequent bone regeneration.[12] However, this theory does not take into account the mechanism of action of these growth factors stored in the platelets. In particular, it has been reported that TGF-β can have either an anabolic or a catabolic effect, depending on the context set by other growth factors present in the wound environment. The anabolic action promotes angiogenesis, chemotaxis and mitogenesis of osteoblast precursors, production of fibronectin, glycosaminoglycans, and collagen in connective tissue.[39–41] Conversely, TGF-β can also present an antiproliferative effect because of its ability to antagonize the mitogenic influences of other peptide growth factors such as PDEGF and PDGF.[41] Furthermore, TGF-β can stimulate the growth of subpopulations of fibroblasts in vitro in the presence of PDGF but inhibits their growth if PDEGF is present.[42] Therefore, the intimate mechanism of growth factor action contained in PRP needs to be studied in more detail before a clinical therapy using this biologic material can be proposed.

Exogenous growth factors are known to act for very short periods of time during the healing cycle. In the current study, the addition of PRP to DFDBG provided no significant increase in BMD or BMC, while the use of the grafting material DFDBG did demonstrate an improvement in BMD and BMC over the negative control. It is possible that the healing times used in this study may have exceeded the times during which PRP would have provided a therapeutic benefit. All implants demonstrated signs of clinical osseointegration at the time of sacrifice. The use or non-use of grafting materials did not appear to have an effect on the ability of the implant to achieve osseointegration. Rather, grafts are used to provide more favorable osseous contours adjacent to implants. This situation is particularly critical when implants are placed in esthetic zones and when bone volume is insufficient to allow full coverage of all alloplastic implant material. Failure of bone to completely cover an implant could result in unaesthetic exposure of the implant. Materials that improve the reliability of bone coverage are beneficial to the clinician; hence the rationale for the suggested use of PRP.

The correlation of BMD and BMC to clinical factors such as immobility has yet to be established. It was previously reported that a potential drawback that could affect the precision of BMD measurements is the repositioning of the ROI after each measurement with DEXA.[43] In that study, the average coefficient of variation with repositioning of the samples was in the range of 5% of mesial and distal sites. It was concluded that small differences in the size and position of the ROI may have resulted in increased variability

in the BMD measurements. To avoid this problem, in the present study all the samples were subjected to a series of 3 repeated measurements of each region without repositioning.

CONCLUSION

In conclusion, the results of this investigation showed no significant differences between regenerative response in the BMD and BMC levels among grafting therapies utilizing PRP in combination with xenogeneic DFDBG and xenogeneic DFDBG alone across all 3 healing periods studied in 9 dogs.

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