Evaluation of Platelet-Rich Plasma in Combination with Anorganic Bovine Bone in the Rabbit Cranium: A Pilot Study

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Purpose: *Platelet-rich plasma (PRP) is potentially useful as an adjunct to allograft and xenograft materials in oral and maxillofacial bone and implant reconstructive surgery. This study compares bone healing and formation in 4 cranial defects in rabbits grafted with autogenous bone, xenograft, and xenograft with PRP (with a no-graft group as a control).* ***Materials and Methods:*** *Fifteen New Zealand white rabbits were included in this randomized, blind, prospective pilot study. Four identical 8-mm-diameter defects were created in each rabbit cranium and immediately grafted with the above materials. Five rabbits were evaluated at 1 month, 5 at 2 months, and 5 at 4 months. Radiographs were used to evaluate bone density.* ***Results:*** *Radiographically, sites at which Bio-Oss, autogenous bone, and Bio-Oss + PRP were grafted showed a significant increase in bone density at 1 month (*$P = .05$ *for Bio-Oss,* $P = .02$ *for autogenous bone,* $P = .008$ *for Bio-Oss + PRP) and at 4 months (*$P = .02$ *for Bio-Oss,* $P = .04$ *for autogenous bone,* $P = .05$ *for Bio-Oss + PRP). Autogenous bone sites (*$P < .001$*) and Bio-Oss + PRP sites (*$P < .001$*) also showed significant increases at 2 months. Histomorphometrically, autogenous bone sites showed a significantly greater increase than control sites (*$P = .08$ *at 1 month,* $P = .03$ *at 2 months,* $P = .01$ *at 4 months), Bio-Oss sites (*$P < .001$ *at all 3 evaluation points), or Bio-Oss + PRP sites (*$P = .009$ *at 1 month,* $P = .02$ *at 2 months,* $P = .01$ *at 4 months). Furthermore, Bio-Oss + PRP sites showed a significantly greater increase in bone area at 1, 2, and 4 months than Bio-Oss alone (*$P = .003$ *at 1 month,* $P = .02$ *at 2 months,* $P = .006$ *at 4 months).* ***Discussion:*** *Radiographs showed significantly greater bone density at the Bio-Oss, autogenous bone, and Bio-Oss + PRP sites than at control sites at nearly every point in time evaluated; however, clinical significance is difficult to determine, since all materials appeared dense on the radiograph. Histomorphometry showed that the increase in bone area at autogenous sites was significantly greater than that seen with other grafting materials or at the control sites.* ***Conclusion:*** *This study showed a histomorphometric increase in bone formation with the addition of PRP to Bio-Oss in non–critical-sized defects in the rabbit cranium.* INT J ORAL MAXILLOFAC IMPLANTS 2004;19:59–65

Key words: *bone grafting, bone substitutes, bovine bone, cranial defects, platelet-rich plasma*

Alveolar bone resorption is a major problem in oral and maxillofacial rehabilitation with dental implants. Bone resorption around teeth results in tooth loss and creates a compromised situation for restoration with osseointegrated implants. Bone grafting is a common surgical procedure performed to regenerate resorbed alveolar bone. Autogenous bone is considered the "gold standard" for grafting oral bony defects.[1,2] However, the required amount of autogenous bone may not always be available, and bone graft harvesting is not without complications and disadvantages, such as the need for a secondary surgical donor site, increased postoperative morbidity, and increased surgical time.

Surgeons are always searching for nonautogenous materials to substitute for autogenous bone. An ideal bone grafting material must be able to produce bone by means of viable transplanted osteoblasts, by osteoinduction of mesenchymal cells, or by osteoconduction along the cell surface. It must maintain mature bone over time without loss of function and provide a low risk for infection and antigenicity. It must have the capability to

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incorporate into the host bone and be available in unlimited quantities.[3,4] Furthermore, its structure must stabilize implants.

Numerous studies have shown the success of anorganic bovine bone grafting materials, but the parameters of success often differ.[5–11] Clinicians are constantly attempting to improve the results obtained with anorganic bovine bone alone by adding barrier membranes,[12,13] fibrin glue,[7,14,15] platelet concentrates,[16] autogenous bone,[17–19] osteogenic protein (BMP-7/OP-1),[20] and other nonautogenous grafting materials.[21]

Bio-Oss is natural bovine bone that is completely deproteinized to prevent a potential immune response.[22,23] Electron microscopic evaluation shows that this material has a structural configuration similar to human bone. Its compressive strength and modulus of elasticity are also similar to the values for human bone.[15,24] It has been shown in numerous studies to have osteoconductive properties; ie, it provides a scaffold on which bone may form.[5,19,25–28] (Osteoconduction, which permits bone apposition from existing bone, requires the presence of bone or differentiated mesenchymal cells.[29,30]) There is no evidence that Bio-Oss is osteoinductive, but it does tend to enable bone healing.[5,27] Nonintegrated bovine material is resorbed by osteoclastic cells.[5] Platelet-rich plasma (PRP) is potentially useful as an adjunct to allograft and xenograft materials in oral and maxillofacial bone and implant reconstructive surgery. Kim and coworkers suggest that the addition of PRP to osteoconductive grafting materials can potentiate osteoinduction.[3]

Platelets are very important in the wound healing process. They quickly arrive at the wound site and begin the coagulation process. They release multiple wound healing growth factors and cytokines, including platelet-derived growth factor (PDGF), transforming growth factors β-1 and β-2 (TGF-β_1 and TGF-β_2), vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor, interleukin-1, basic fibroblast growth factor, and platelet activating factor-4.[31–35] These growth factors are thought to contribute to bone regeneration and increased vascularity, vital features of a healing bone graft.

We do not know whether PRP can be used with alloplasts, xenografts, or other nonautogenous materials without the addition of autogenous donor bone to create a bone graft comparable to or superior to autogenous bone. There are very few studies in refereed journals in which PRP was added to allograft or alloplast bone.[3,36–38] These articles describe the PRP techniques used and reported the formation of some osteoid material or bone. However, the results are conflicting or equivocal.[36,37,39,40] In many of these studies, few cases were evaluated and no statistical testing was performed to confirm the validity of the results.[36–40] Scientific conclusions cannot be reached based on these limited studies.

Further scientific testing of PRP in combination with xenograft materials such as anorganic bovine bone is obviously necessary. The present study was designed to test the effectiveness of PRP when added to a xenograft.

MATERIALS AND METHODS

Animal Surgical Procedure

Fifteen New Zealand white male rabbits weighing between 2.8 and 4 kg were included in this randomized, blind, prospective pilot study. The University of California at Los Angeles (UCLA) Animal Research Committee, in accordance with staff in the UCLA Department of Laboratory and Animal Medicine, approved all animal protocols. Each rabbit was anesthetized with ketamine hydrochloride (10 mg/kg) and acepromazine (1 to 1.5 mg/kg) and given enrofloxacin as a preoperative antibiotic (Baytril, 5 mg/kg, Bayer, Shawnee Mission, KS). To prepare the PRP, 10 mL of autologous blood was drawn from each rabbit. The rabbits underwent general anesthesia with 1% to 2% isoflurane with standard monitoring. The fur was shaved over the cranium and prepped and draped in a sterile fashion. An incision was made to the bony cranium, and the periosteum was reflected. Four 8-mm-diameter defects were created with a trephine bur using copious irrigation. Three of the defects were randomly grafted with autogenous bone alone (obtained by particulating the trephinated bone), Bio-Oss mixed with PRP, or Bio-Oss alone. As a control, the fourth defect was not grafted. The wound was closed with 3-0 Dexon (Owens and Minor, Irvine, CA) and the rabbits were recovered. They were given postoperative narcotic pain medication and antibiotics.

PRP Preparation

The 10 mL of autologous blood drawn from each rabbit was combined with 1.1 mL of anticoagulant citrate dextrose phosphate (ACD-A) to prevent coagulation. The blood was spun in a centrifuge (Sorvall, Kendro Laboratory Products, Newtown, CT) at 1,500 rpm (215 g) for 10 minutes to separate the plasma containing the platelets from the red blood cells. The plasma was drawn off the top of the test tube, mixed with 0.4 mL of ACD-A, and centrifuged for an additional 10 minutes at 3,000 rpm (863 g) to separate the platelets. The platelet-poor plasma

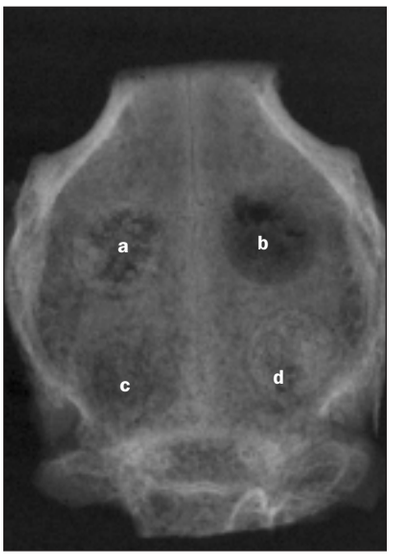
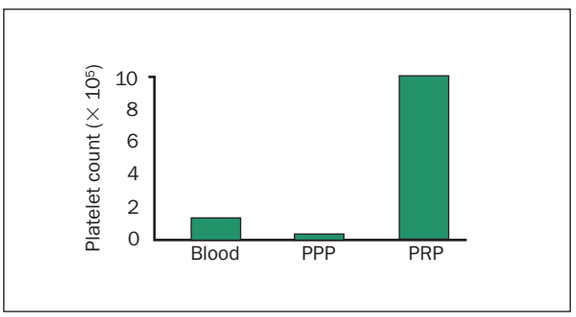


Fig 1 *(Left)* Radiograph of a rabbit cranium harvested after 2 months of healing: (a) Bio-Oss, (b) control, (c) autogenous bone, (d) Bio-Oss + PRP.

Fig 2 *(Below)* Average platelet counts in peripheral blood, PPP, and PRP. Platelet counts confirmed that PRP preparation produced highly concentrated platelets.



(PPP) was separated from the PRP and the buffy coat. The buffy coat and the PRP, approximately 1 to 1.5 mL, were resuspended and added to the grafting materials within minutes. Five thousand units of topical bovine thrombin powder (King, St Louis, MO) was reconstituted with 5 mL of 10% calcium chloride (American Regent, Shirley, NY). The ratio of PRP to calcium chloride activator was 10:1. This protocol is similar to that utilized in clinical practice with some of the commercially available machines and described in an article by Jones and coworkers.[34]

Platelet counts were performed on each sample, including a peripheral blood count, a PPP count, and a PRP count. A Unopette microcollection system (Becton Dickinson, Franklin Lanes, NJ) was used to lyse the leukocytes and erythrocytes, as well as provide a standard dilution of 1:100 for platelet counts. The platelets were hand counted with a standard hemocytometer, and the total was calculated for each sample.

Sample Evaluation

The rabbits were divided into 3 groups of 5. One group was sacrificed at 1 month, one at 2 months, and one at 4 months. Pentobarbital (100 mg/kg intravenously) was used for this purpose. The entire cranium was removed with a reciprocating saw without encroaching upon the grafted areas.

Radiographs were taken of the rabbit cranium in its entirety before histologic sectioning was performed (Fig 1). A calcium carbonate step-wedge was used in each radiograph for comparison. Quantification was performed with digital subtraction radiography. Computer software (UCLA Image, Los Angeles, CA) compared the pixels and grams per square inch of all 4 sites for each rabbit.

Specimens were treated with hydrochloric acid decalcifying solution (Fisher Scientific, Tustin, CA) and sectioned by bisecting the 8-mm-diameter defects. Specimens were then dehydrated with graded alcohols and embedded in paraffin. They were subsequently sectioned at 6 µm with a steel knife. These 6-µm sections were prepared in the usual fashion with hematoxylin and eosin staining. Histologic evaluation was performed at 10×, 25×, and 40× magnification. The 40× magnification was used for histomorphometric analysis. The bone area was calculated using ImagePro software (Media Cybernetics, Silver Springs, MD). Evaluators were blind to the grafting material and time period for each sample. The data were analyzed by a Student *t* test with a significance level at $P \leq .05$.

RESULTS

Platelet counts confirmed that the PRP preparation technique used in this study produced a highly concentrated source of platelets (Fig 2). The average peripheral blood platelet count was 118,000/mm^3, with a range of 100,000 to 135,000/mm^3. The average platelet count in PPP was 22,330/mm^3, with a range of 15,000 to 30,000/mm^3. The average platelet count in PRP was 960,000/mm^3, with a range from 660,000 to 1,265,000/mm^3.

Radiographic Evaluation

Figure 3 demonstrates the bone density as determined radiographically. Autogenous bone ($P = .02$ at 1 month, $P = .001$ at 2 months, $P = .04$ at 4 months) and Bio-Oss + PRP ($P = .08$ at 1 month, $P = .001$ at 2 months, $P = .05$ at 4 months) showed a significant

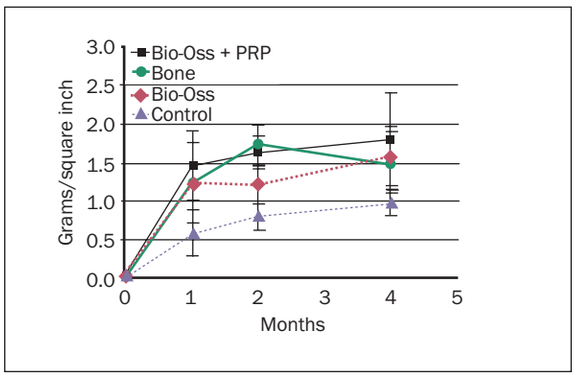


Fig 3 Amount of bone fill, determined radiographically over the 4-month study period.

increase in radiographic bone density when compared to the control at all 3 time points. Bio-Oss alone was significantly better than the control at 1 month (P = .05) and 4 months (P = .02) but not at 2 months. However, at no time was there a statistically significant difference in radiographic density when Bio-Oss alone was compared to Bio-Oss + PRP. All statistical analyses were performed utilizing the mean and standard errors to determine significance.

Histologic Evaluation

Figure 4 shows the histologic evaluation of all grafting materials at 1 and 4 months at 10× magnification. Fibrous tissue, which filled the defect gradually over the 4 months, was minimal at 1 month. Bio-Oss sites showed Bio-Oss particles surrounded by fibrous connective tissue, which underwent remodeling at 1 month, with minimal bony ingrowth by 4 months. Autogenous bone graft material mixed with fibrous connective tissue was seen at 1 month. The graft material underwent remodeling and became significantly more dense by 4 months. Bio-Oss + PRP at 1 month showed fibrous connective tissue with Bio-Oss particles surrounded by bony ingrowth. This bone consolidated, and few Bio-Oss particles were seen by 4 months.

Histomorphometric Evaluation

Figure 5 shows the histomorphometric percent bone area as a function of time for each grafting material. Autogenous bone graft showed a significantly greater bone area when compared with each of the other grafting materials at each time point. Only autogenous bone graft showed a statistically significant increase in bone area histomorphometrically compared to all 3 other sites at all 3 evaluation points (for control sites, P = .08, P = .03, P = .01, at 1, 2, and 4 months, respectively; for Bio-Oss, $P < .001$ at all 3 evaluation points; for Bio-Oss + PRP, P = .009, P = .02, P = .01, at 1, 2, and 4 months, respectively).

When Bio-Oss + PRP was evaluated histomorphometrically, there was a statistically significant increase in bone area at 1, 2, and 4 months compared to Bio-Oss alone (P = .003, P = .02, P = .006, respectively). Bio-Oss alone actually showed a histomorphometric tendency toward decreased bone area at 1, 2, and 4 months when compared to the control, but this was not statistically significant ($P > .05$).

DISCUSSION

Oral and maxillofacial surgery researchers strive continuously to improve upon current bone-grafting techniques and provide faster and denser bony regeneration. Growth factors are a realistic way to improve and expedite both soft tissue and bony wound healing. Platelets contain angiogenic, mitogenic, and vascular growth factors in their granules,[41,42] such as VEGF, a powerful angiogenic growth factor with an important role in wound healing[43]; TGF-β_1 and TGF-β_2, which have been shown to inhibit bone resorption, osteoclast formation, and osteoclast activity, as well as trigger rapid maturation of collagen in early wounds[44,45]; and PDGF, which increases the population of wound-healing cells and recruits other angiogenic growth factors to the wound site.[45] It is therefore a reasonable hypothesis that increasing the concentration of platelets in a bone defect may lead to improved and faster healing. However, little evidence exists evaluating the ability of these growth factors to improve bone healing when added to osteoconductive materials.[3,16]

Quantitative platelet counts verified that PRP, consisting of 660,000 to 1,265,000 platelets in 1 to 1.5 mL of concentrate,[46] was used in this study. Digital subtraction radiography with step-wedge calibration showed a significant increase in bone density when Bio-Oss, autogenous bone, and Bio-Oss + PRP were grafted, compared to ungrafted controls, at nearly every evaluation point. The clinical significance of these data is difficult to determine because any radiopaque bone grafting material will look more dense on a radiograph. No significant difference could be seen between the 3 grafting materials radiographically. These results conflict with those of Kim and associates, who showed significantly greater bone density at 1 and 2 months when evaluating digitized radiographs and computed tomography scans when PRP was added to Bio-Oss in rabbit cranial defects.[16]

When histologic sections were evaluated with histomorphometry, autogenous bone graft demonstrated a statistically significant increase in bone

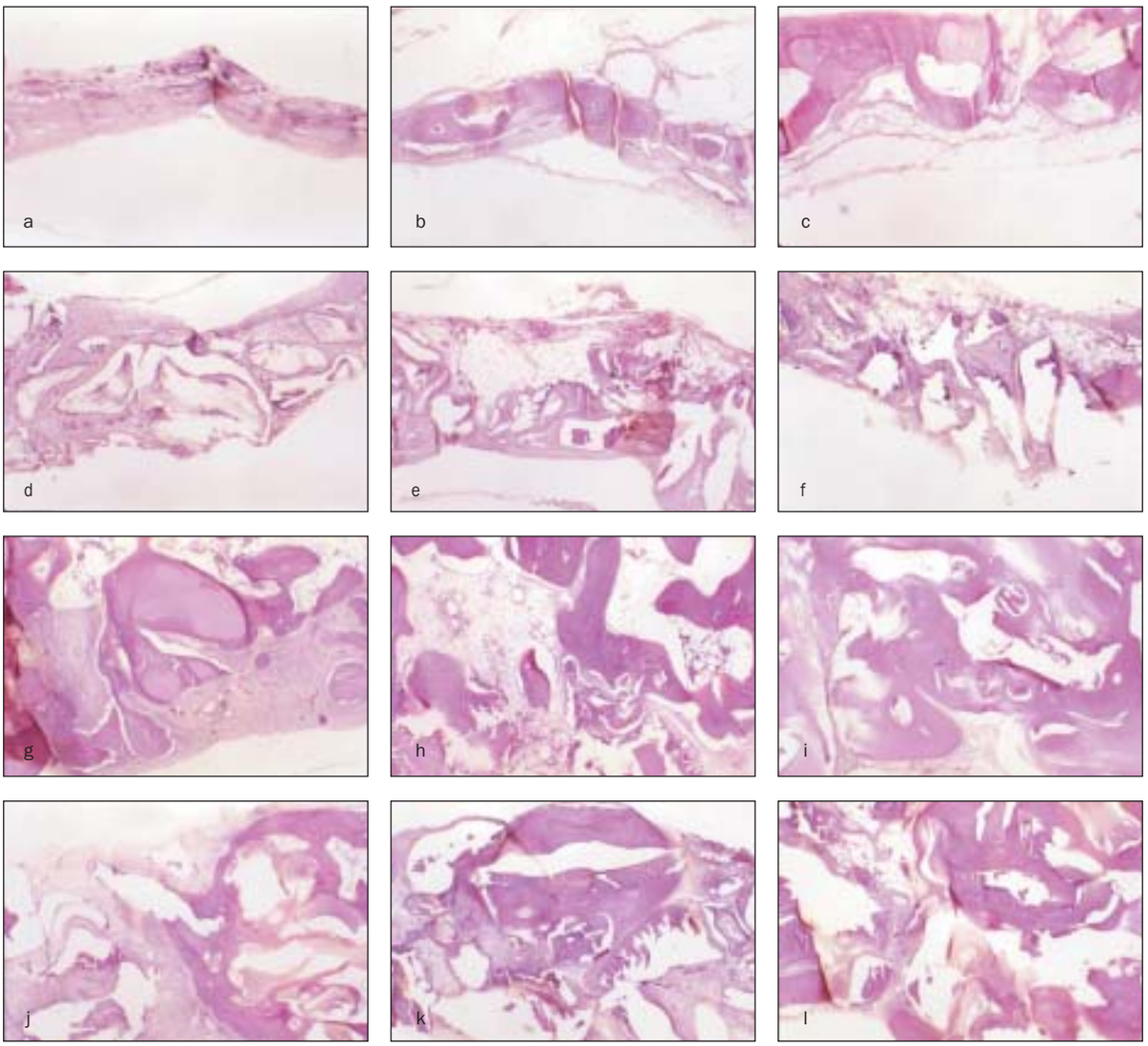


Fig 4 Histologic evaluation of all grafted materials at 1, 2, and 4 months. Magnification 40×: *(a)* control, 1 month; *(b)* control, 2 months; *(c)* control, 4 months; *(d)* Bio-Oss, 1 month; *(e)* Bio-Oss, 2 months; *(f)* Bio-Oss, 4 months; *(g)* autogenous bone, 1 month; *(h)* autogenous bone, 2 months; *(i)* autogenous bone, 4 months; *(j)* Bio-Oss + PRP, 1 month; *(k)* Bio-Oss + PRP, 2 months; *(l)* Bio-Oss + PRP, 4 months.

area over the control, Bio-Oss alone, and Bio-Oss + PRP at 1, 2, and 4 months. No other animal or human studies that have compared Bio-Oss + PRP to autogenous bone grafting are currently available. Adding PRP to Bio-Oss resulted in a significant increase in bone area at all time periods compared with Bio-Oss alone. These data are not in agreement with the results of Froum and associates.[38] In that study, human sinus-grafted core samples were evaluated histomorphometrically after grafting with Bio-Oss alone or Bio-Oss + PRP. The authors found no histologic benefit by the addition of PRP. They did not use radiographic comparison in their study.

Other preliminary reports have shown that freeze-dried demineralized bone combined with PRP forms numerous areas of osteoid and bone without inflammatory infiltrate or soft tissue epithelialization, with osseous trabeculae surrounding the connective tissue.[36,37] However, quantitative analysis was not performed in these studies, and the grafted sites were not standardized or randomized.

When the Bio-Oss alone sites were analyzed, a histomorphometric tendency toward decreased bone area was observed at 1, 2, and 4 months compared to control, but this was not statistically significant. As stated in the introduction, this product has been shown in numerous other studies to have osteoconductive properties, where it provides a scaffold onto which bone may form.[5,19,25–28]

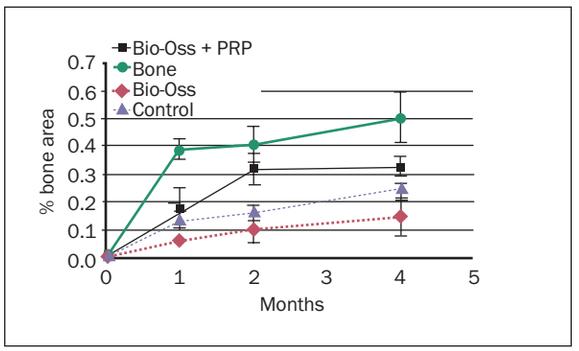


Fig 5 Histomorphometric evaluation of bone area over the 4-month study period.

In this study, the addition of PRP to Bio-Oss in the rabbit cranial defect model was shown to be potentially beneficial. However, autogenous bone alone still showed the greatest histomorphometric bone area at all evaluation points. Furthermore, the sample was small, consisting of only 15 rabbits.

Further studies are needed to evaluate the potential benefits of PRP in combination with various autogenous, allograft, and alloplast grafting materials. Four critical-sized defects (critical size being 15 mm wide[47]) cannot be created in the rabbit cranium because it is too small. Therefore, this study was designed to evaluated non–critical-sized cranial defects. However, future studies need to evaluate the potential benefits of PRP in healing critical-sized defects.

CONCLUSION

This study evaluated grafting materials in rabbit cranial defects, and showed significant histomorphometric improvement at 1, 2, and 4 months with the addition of PRP to Bio-Oss. However, when autogenous bone was used in the same model, the results were still superior to the ungrafted control, Bio-Oss alone, and Bio-Oss + PRP at all evaluation points.

REFERENCES

1. Moy PK, Lundgren S, Holmes RE. Maxillary sinus augmentation: Histomorphometric analysis of graft materials for maxillary sinus floor augmentation. J Oral Maxillofac Surg 1993;51:857–862.
2. Gross JS. Bone grafting materials for dental applications: A practical guide. Compend Contin Educ Dent 1997;18: 1013–1018, 1020–1022, 1024.
3. Kim SG, Chung CH, Kim YK, Park JC, Lim SC. Use of particulate dentin-Plaster of Paris combination with/without platelet-rich plasma in the treatment of bone defects around implants. Int J Oral Maxillofac Implants 2002;17:86–94.
4. Block MS, Kent JN. Sinus augmentation for dental implants: The use of autogenous bone. J Oral Maxillofac Surg 1997; 55:1251–1261.
5. Merkx MAW, Maltha JC, Freihofer HPM, Kuijpers-Jagtman AM. Incorporation of three types of bone block implants in the facial skeleton. Biomaterials 1999;20:639–645.
6. Carmagnola D, Berglundh T, Araujo M, Albrektsson T, Lindhe J. Bone healing around implants placed in a jaw defect augmented with Bio-Oss: An experimental study in dogs. J Clin Periodontol 2000;27:799–805.
7. Carmagnola D, Berglundh T, Lindhe J. The effect of fibrin glue on the integration of Bio-Oss with bone tissue: An experimental study in labrador dogs. J Clin Periodontol2002;29:377–381.
8. Mayfield LA, Skoglund A, Hising P, Lang NP, Attstrom R. Evaluation following functional loading of titanium fixtures placed in ridges augmented by deproteinized bone mineral: A human case study. Clin Oral Implants Res 2001;12:508–514.
9. Zitzmann NU, Scharer P, Marinello CP, Schupbach P, Berglundh T. Alveolar ridge augmentation with Bio-Oss: A histologic study in humans. Int J Periodontics Restorative Dent 2001;21:288–295.
10. van Steenberghe D, Callens A, Geers L, Jacobs R. The clinical use of deproteinized bovine bone mineral on bone regeneration in conjunction with immediate implant installation. Clin Oral Implants Res 2000;11:210–216.
11. Tal H. Autogenous masticatory mucosal grafts in extraction socket seal procedures: A comparison between sockets grafted with demineralized freeze-dried bone and deproteinized bovine bone material. Clin Oral Implants Res 1999;10:289–296.
12. Houser BE, Mellonig JT, Brunsvold MA, Cochran DL, Meffert RM, Alder ME. Clinical evaluation of anorganic bovine bone xenograft with a bioabsorbable collagen barrier in the treatment of molar furcation defects. Int J Periodontics Restorative Dent 2001;21:161–169.
13. Hämmerle CH, Chiantella GC, Karring T, Lang NP. The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. Clin Oral Implants Res 1998;9:151–162.
14. Hallman M, Cederlund A, Lindskog S, Lundgren S, Sennerby L. A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6 to 8 months of healing. Clin Oral Implants Res 2001;12:135–143.
15. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D. Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood: A histologic and histomorphometric study in humans. Clin Oral Implants Res 2000;11:217–229.
16. Kim ES, Park EJ, Choung PH. Platelet concentration and its effect on bone formation in calvarial defects: An experimental study in rabbits. J Prosthet Dent 2001;86:428–433.
17. Maiorana C, Santoro F, Rabagliati M, Salina S. Evaluation of the use of iliac cancellous bone and anorganic bovine bone in the reconstruction of the atrophic maxilla with titanium mesh: A clinical and histologic investigation. Int J Oral Maxillofac Implants 2001;16:427–432.
18. Maiorana C, Redemagni M, Rabagliati M, Salina S. Treatment of maxillary ridge resorption by sinus augmentation with iliac cancellous bone, anorganic bovine bone, and endosseous implants: A clinical and histologic report. Int J Oral Maxillofac Implants 2000;15:873–878.
19. Yildirim M, Spiekermann H, Handt S, Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: A histologic and histomorphometric clinical study in humans. Int J Oral Maxillofac Implants 2001;16:23–33.

20. McAllister BS, Margolin MD, Cogan AG, Taylor M, Wollins J. Residual lateral wall defects following sinus grafting with recombinant human osteogenic protein-1 or Bio-Oss in the chimpanzee. Int J Periodontics Restorative Dent 1998;18:227–239.
21. Su-Gwan K, Hak-Kyun K, Sung-Chul L. Combined implantation of particulate dentine, plaster of Paris, and a bone xenograft (Bio-Oss) for bone regeneration in rats. J Craniomaxillofac Surg 2001;29:282–288.
22. Hislop WS, Finlay PM, Moos KF. A preliminary study into the uses of anorganic bone in oral and maxillofacial surgery. Br J Oral Maxillofac Surg 1993;31:149–153.
23. Indovina A, Block MS. Comparison of 3 bone substitutes in canine extraction sites. J Oral Maxillofac Surg 2002;60:53–58.
24. Thaller SR, Hoyt J, Borieson K, Dart A, Tesluk H. Reconstruction of calvarial defects with anorganic bovine bone mineral (Bio-Oss) in a rabbit model. J Craniofac Surg 1993; 4:79–84.
25. Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials: A histological study in beagle dogs. Clin Oral Implants Res 1995;6:155–163.
26. Valentini P, Abensur D. Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (Bio-Oss): A clinical study of 20 patients. Int J Periodontics Restorative Dent 1997;17:232–241.
27. Klinge B, Alberius P, Isaksson S, Jonsson J. Osseous response to implanted natural bone mineral and synthetic hydroxylapatite ceramics in repair of experimental skull bone defects. J Oral Maxillofac Surg 1992;50:241–249.
28. Hämmerle CH, Olah AJ, Schmid J, et al. The biological effect of natural bone mineral on bone neoformation on the rabbit skull. Clin Oral Implants Res 1997;8:198–207.
29. Masters DH. Implants: Bone and bone substitutes. J Calif Dent Assoc 1988;16:56–65.
30. Misch CE, Dietsh F. Bone-grafting materials in implant dentistry. Implant Dent 1993;2:158–167.
31. Linder BL, Chernoff A, Kaplan KL, Goodman DS. Release of PDGF from human platelets by arachidonic acid. Proc Natl Acad Sci USA 1979;76:4107–4111.
32. Miyadera K, Sumizawa T, Haraguchi M, et al. Role of thymidine phosphorylase activity in the angiogenic effect of PDEGF/thymidine phosphorylase. Cancer Res 1995;55:1687–1690.
33. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of VEGF by human megakaryoctes and platelets. Proc Natl Acad Sci USA 1997;94:663–668.
34. Jones CL, Witte DP, Feller MJ, Fugman DA, Dorn GW II, Lieberman MA. Response of human megakaryocytic cell line to thrombin: Increase in intracellular free calcium and mitogen release. Biochim Biophys Acta 1992;1136:272–282.
35. Harrison P, Cramer EM. Platelet alpha granules. Blood Rev 1993;7:52–62.
36. Kassolis JD, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: Case series. J Periodontol 2000;71:1654–1661.
37. Shanaman R, Filstein MR, Danesh-Meyer MJ. Localized ridge augmentation using GBR and platelet-rich plasma: Case reports. Int J Periodontics Restorative Dent 2001;21:345–355.
38. Froum S, Cho SC, Rosenberg E, Rohrer M, Tarnow D. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: Three bilateral case reports. Int J Periodontics Restorative Dent 2002;22:45–53.
39. Rosenberg ES, Torosian J. Sinus grafting using platelet-rich plasma—Initial case presentation. Pract Periodontics Aesthet Dent 2000;12:843–850.
40. Anitua E. Plasma rich in growth factors: Preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants 1999;14:529–535.
41. Banks RE, Forbes MA, Kinsey SE, et al. Release of the angiogenic cytokine VEGF from platelets: Significance for VEGF measurements and cancer biology. Br J Cancer 1998;77:956–964.
42. Maloney JP, Silliman CC, Ambruso DR, Wang J, Tuder RM, Voelkel NF. In vitro release of vascular endothelial growth factor during platelet aggregation. Am J Physiol 1998;275(3 Pt 2):H1054–H1061.
43. Thomas KA. Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem 1996;271:603–606.
44. Bonewald LF, Mundy GF. Role of TGF-β in bone remodeling. Clin Orthop 1990;250:261–273.
45. Steenfos HH. Growth factors and wound healing. Scand J Plast Reconstr Surg Hand Surg 1994;28:95–105.
46. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol 1998;85:638–646.
47. Vikjaer D, Blom S, Hjorting-Hansen E, Pinholt EM. Effect of platelet-derived growth factor-BB on bone formation in calvarial defects: An experimental study in rabbits. Eur J Oral Sci 1997;105:59–66.