Role of platelet-rich plasma in combination with alloplastic bone substitute in regeneration of osseous defects

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ABSTRACT

Introduction: Bone grafts are frequently used for the treatment of bone defects, but can cause postoperative complications, and sometimes a sufficient quantity of bone is not available. Hence, synthetic biomaterials have been used as an alternative to autogenous bone grafts. Recent clinical reports suggest that application of autologous blood plasma enriched with platelets can enhance the formation of new bone. There are very few *in vitro* or *in vivo* studies published on the efficiency of platelet-rich plasma (PRP). The objective of this study was to evaluate the alloplastic bone substitute for its osteogenic potential with or without PRP.

Materials and Methods: Twenty-three patients with periapical bony defects were selected for this study. Clinical parameters such as pain visual analog scale (VAS), swelling, infection, graft migration, rejection, radiographical interpretations at regular interval and scintigraphic evaluation were done to evaluate osteogenic potential of alloplastic bone substitute with or without PRP.

Results: The highest acceleration in bone formation was observed in groups where alloplastic bone substitute was used with PRP. There were no statistically significant differences between the two groups regarding other outcome variables throughout the postoperative period.

Conclusion: Addition of PRP significantly accelerates vascularization of the graft, improves soft tissue healing, reduces postoperative morbidity and enhances bone regeneration.

Keywords: Bone graft, osseous defects, platelet-rich plasma.

INTRODUCTION

Autogenous bone grafts have been the most accepted and commonly used modality for osseous defects by virtue of osteo-conductivity, osteo-induction, osteogenic potential and lack of immunogenicity,¹ but suffer from limitations namely inadequate amount, donor site morbidity and early resorption of bone. To overcome these problems, alloplastic bone substitutes have been used for bone regeneration as an alternative graft material. Alloplastic bone graft materials are synthetic, inorganic, biocompatible and bioactive bone substitutes that promote healing of bony defects through osteo-conduction but lack cellular components required for osteogenesis.²

Several biomaterials namely hydroxyapatite (HA) and tricalcium phosphate (TCP) have been tested for tissue compatibility and have not elicited any inflammation or foreign body reaction. Hydroxyapatite does not stimulate osteogenesis, but is osteo-conductive when placed next to

viable bone, as new bone grows into the porous matrix.³ The present trend, however, in maxillofacial reconstructive surgery is the introduction and usage of platelet concentrates to promote bone formation and to accelerate the bone graft maturation.

The use of platelet-rich plasma (PRP) is based on the premise that large number of platelets in PRP release significant quantities of growth factors that aid bone graft maturation.⁴

MATERIALS AND METHODS

Twenty-three patients with periradicular bony defect, attending the outpatient department (OPD) clinic at the Department of Oral and Maxillofacial Surgery, UP Dental College and Research Center, Lucknow, were included in the study, irrespective of cast, creed, sex and socio-economic status.

The study design and consent were approved by the institutional ethical committee. The treatment plan was thoroughly explained, and standard consent was obtained. After recording a detailed history, patients were thoroughly examined and routine hematological investigations were carried out. Pre-operative radiographical evaluation of the area was done on standard intra-oral periapical radiographs of the region concerned. Patients suffering from any systemic diseases, pregnant and lactating mothers were excluded from the study.

G-Bone Synthetic composed of synthetic TCP and HA of average particle size 0.8–1.8 mm (Surgiwear™) was used as alloplastic bone substitute (ABS). It has a porous structure with parallel channels and interconnecting fenestrations and a macroscopic architecture resembling that of human cancellous bone. Patients were randomly divided into two groups: Group A had 10 (43.5%) patients whose osseous defects were filled with ABS mixed in normal saline (NS) and Group B had 13 (56.5%) patients whose osseous defects were filled with ABS with PRP.

The PRP was prepared in the Department of Pharmacy, Babu Banarasi Das National Institute of Technology and Management, Lucknow by centrifugation immediately before the operation. Patient's 10 mL blood was drawn and collected into the sterile tubes containing about 2 mg ethylene diamine tetra acetate as anticoagulant. The content in the tube was mixed well before placing into the centrifuge. The first centrifugation was done at 2400 rpm for 10 min. This procedure divided the blood into three basic components: red blood cells at the lowest level, buffy coat-PRP in the center and straw-colored platelet-poor plasma (PPP) at the top. The PPP, PRP and the top part of the red blood cells was aspirated and transferred into the second tube and a second centrifugation was performed at 3600 rpm for 15 min. The platelet concentrated at the bottom of the tube, whereas PPP concentrated on top and was removed so that PRP remained in the tube. At the time of the use, PRP was mixed manually with ABS granules before being placed into the bony defect.

The procedure was carried out under local anesthesia. A trapezoidal incision for apicoectomy was made for reflection of the mucoperiosteal flap, and all granulation tissue/cyst was curetted from the defect. The apex of the tooth/teeth involved were sectioned and removed. Wound was irrigated with povidine-iodine. Obturation of tooth/teeth was done using guttapercha points and zinc oxide eugenol. The osseous defects were filled according to their group, the flap was repositioned and sutured (Figure 1).

Patients were monitored postoperative at days 1, 7, 30, 90 and 180 for postoperative pain (VAS), swelling, infection, change in color of mucosa, pus discharge, lymph nodes,

graft migration, rejection (discharge/extrusion/flap dehiscence), and radiographical evaluation of graft incorporation as per Irwin's radiological staging (Tables 1 and 2) using standard intra-oral periapical radiographs at all the postoperative follow-ups (Figure 2).

Three patients were subjected to a three-phase bone scan after i.v. injection of 20 mCi of 99m-Tc methylene diphosphonate (MDP) where early perfusion, delayed perfusion and blood pool images were acquired (Figure 3). The single photon emission computed tomography (SPECT) was done subsequently with low-dose 2.5-mA X-rays. These 3 patients had bilateral periradicular defects in which one of the osseous defects was filled using ABS with PRP and the other osseous defect was filled using ABS with NS. Subsequently, they were additionally evaluated using SPECT imaging.

RESULTS

Healing was uneventful in all the patients except two, where the graft became infected and was lost due to wound dehiscence. Significantly higher proportion of Group B (ABS + PRP) subjects had hazy and incorporated graft margins as compared with Group A (ABS + NS).

All patients in both the groups had mild pain on day 1. However, on week 1, only 6 patients, 4 (40%) in Group A and 2 (15.4%) in Group B had pain. At 1 and 3 months, only 1 (7.7%) patient in Group B had pain. Statistically, there was no significant difference between two groups at any time interval ($P > 0.05$).

All patients in both groups had mild postoperative swelling. However, on day 7, only 5 patients, 4 (40%) in Group A and 1 (7.7%) in Group B had swelling. At 1 and 3 months, only 1 patient of Group B (7.7%) had swelling. Statistically, there was no significant difference between two groups at any time interval ($P > 0.05$).

Infection was observed in 1 patient at 1 and 3 months in Group B, while the other patient with infection at 3 months was excluded from further study owing to rejection of graft. There was no graft migration in any of the patients during the study period except at 1 month in 1 patient in Group B. However, statistically, there was no significant difference between two groups at any time interval. Overall proportion of graft rejection was 4.3%.

Disappearance of radiolucent zone between the bone cavity and the graft was early and in significantly higher proportion of patients in Group B as compared with Group A. The onset of intrinsic graft indistinctiveness was early and in significantly higher proportion of patients in Group B as compared with Group A.

Figure 1 (A) Incision, (B) osseous defect, (C) alloplastic bone substitute mixed with saline, (D) alloplastic bone substitute mixed with platelet-rich plasma, (E) graft placed in defect, and (F) closure.

At days 1 and 7, all patients had obvious margins in both the groups. Significantly higher proportion of Group B patients had hazy margins as compared with Group A at 1 month. Proportion of patients with hazy and incorporated margins were significantly higher in Group B (ABS+PRP)

as compared with Group A (ABS+NS) ($P < 0.001$) at 3 or 6 months.

The resolution of radiolucency disappeared early in significantly higher proportion of patients in Group B as compared with Group A. Significant differences between

two groups were observed from day 7 onwards. All but 1 patient in Group B showed no radiolucency, whereas in Group A, in only 1 of the 10 patients, the radiolucency was found to be absent ($P < 0.001$).

The onset of graft indistinctiveness was early in significantly higher proportion of patients in Group B as compared with Group A. Significant differences between two groups were observed from 1 month onwards. In all but 2 patients in Group B, the graft fully disappeared at 6 months follow-up, whereas in Group A, all the 10 patients had indistinct graft which did not disappear ($P < 0.001$).

Table 1 Irwin's radiological stages of graft incorporation

Stage	Radiolucent zone between the bone cavity and the graft	Intrinsic graft indistinctiveness	Graft margins
I	Present	Indistinct	Obvious
II	Indistinct	Indistinct	Hazy
III	Indistinct/disappearance	Indistinct/disappearance	Obvious incorporation

The scintigraphic findings of 3 patients with bilateral defects showed increased tracer uptake in region of osseous defect filled with ABS with PRP. The tracer uptake was 1.36 times higher in 1 patient, 1.30 times in second and 1.79 times in third patient in region of osseous defect filled with ABS with PRP.

DISCUSSION

Bone is a dynamic tissue that shows marked structural alteration in response to injury, stress, vascular, endocrine, genetic and nutritional influences.⁵ Management of bony defects often poses problems during grafting. These problems have been partially resolved with the advent of newer substitutes. Alloplasts are biocompatible osteo-conductive materials, used to replace human bone.⁶ In this process, vascular tissue invades the graft, bringing with it the osteoblasts that deposit new bone, hence called 'creeping substitution' and represents osteo-conduction.⁵ Both HA and

Table 2 Radiographical assessment at different time intervals

		Group A (n=10)			Group B (n=13)*			Total (n=23)			Significance of difference	
		O	H	I	O	H	I	O	H	I	z	P
Graft margins	Time interval											
1	Day 1	10	0	0	13	0	0	23	0	0	0	1
2	Day 7	10	0	0	13	0	0	23	0	0	0	1
3	Day 30	10	0	0	2	11	0	12	11	0	3.939	<0.001
4	Day 90	3	7	0	0	2	10	3	9	10	3.820	<0.001
5	Day 180	0	10	0	0	0	12	0	10	12	4.583	<0.001
Radiolucent zones	Time interval	P	I	D	P	I	D	P	I	D	z	P
1	Day 1	10	0	0	2	11	0	12	11	0	1.270	0.563
2	Day 7	10	0	0	4	9	0	14	9	0	3.298	0.004
3	Day 30	10	0	0	0	9	4	10	9	4	4.361	<0.001
4	Day 90	0	9	1	0	1	11	0	10	12	3.965	<0.001
5	Day 180	0	9	1	0	1	11	0	10	12	3.965	<0.001
Graft indistinctiveness	Time interval	P	I	D	P	I	D	P	I	D	z	P
1	Day 1	10	0	0	12	1	0	22	1	0	0.877	0.784
2	Day 7	10	0	0	10	3	0	20	3	0	1.593	0.376
3	Day 30	9	1	0	4	6	3	13	7	3	2.790	0.012
4	Day 90	8	2	0	0	8	4	8	10	4	3.711	<0.001
5	Day 180	0	10	0	0	2	10	0	12	10	3.919	<0.001

(Mann-Whitney U-test); *One patient in Group B was lost to follow-up at day 180. O=obvious; H=hazy; I=incorporated; P=present; I=indistinct; D=disappeared.

Figure 2 (A) Pre-operative: A1 & A2, peri-radicular lesion, (B) postoperative at first day: B1, alloplastic bone substitute + NS and B2, – alloplastic bone substitute + platelet-rich plasma and (C) postoperative at 6th month: C1, alloplastic bone substitute + normal saline and C2, alloplastic bone substitute + platelet-rich plasma.

TCP in their ceramic form act as excellent bone graft substitute in filling bone voids after curettage of benign bone tumors.¹ The ABS when placed in contact with living bone causes no tissue reaction, rapidly consolidates with the host bone and incorporates into the bony framework of face.

Platelet-rich plasma was first introduced to the oral and maxillofacial surgery community by Whitman et al in 1997 through an article entitled 'Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery'.⁷ The PRP is known to contain a number

Figure 3 Scintigraphic evaluation of 3 patients (1, 2 and 3) increased the tracer uptake in region of osseous defect filled with alloplastic bone substitute mixed with platelet-rich plasma.

of growth factors/cytokines that may aid in the accelerated maturation of a bone graft.⁸

The first study on the use of PRP reported an addition of PRP to an autogenous cancellous bone graft resulting in faster maturation rate and greater amount of new bone formation in an alveolar defect in addition to significantly greater bone density.⁹ The PRP enhances osteo-progenitor cells in the host bone and in bone grafts thereby inducing osteogenic effect.¹⁰ In addition PRP improves the rate and quality of vascular in-growth, increases bone regeneration, enhances soft tissue healing and exerts mitogenic effects on critical cells.¹¹ Contrary to this, in another study, activated PRP had no significant effect on the early cortical bone response to the Ca-P-coated implants while PRP in a liquid form without any activation showed a tendency to increase bone apposition to roughened titanium implants.¹²

Three patients on scintigraphic assessment showed significant increased tracer uptake in region of osseous defect filled using ABS with PRP. Additionally, there was earlier

consolidation of the graft in Group B in these cases. The reason can be attributed to large number of platelets in PRP releasing significant quantities of growth factors that aid bone graft maturation. The influential time of the growth factors of the PRP is up to 6–7 months.¹³

In our study, the consolidation of the graft was earlier in Group B patients in whom the ABS was mixed with PRP which was based on the premise that the large number of platelets in PRP release significant quantities of growth factors that aid bone graft maturation in accordance with Consolo et al,¹³ Marx,¹⁴ Green David and Klink¹⁵ and Messori et al,¹⁶ but contradictory to Shanaman et al,¹⁷ Arpornmaeklong et al,¹⁸ de Vasconcelos et al¹⁹ and Harris et al.²⁰

CONCLUSION

Platelet-rich plasma accelerates vascularization of the graft, improves soft tissue healing, reduces postoperative morbidity

and enhances bone regeneration. Advantages of using an autologous PRP include no risk of cross-reactivity, immune reaction or disease transmission. In addition, the use of PRP improves handling characteristics of particulate graft material and affords easier packing into a grafting site thus, facilitating space maintenance and potential for bone regeneration.

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