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COMPARATIVE STUDY

Potential for osseous regeneration of platelet rich plasma: a comparitive study in mandibular third molar sockets

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Abstract
Purpose To evaluate the efficacy of autologous platelet- rich plasma in soft tissue healing & bone regeneration in mandibular third molar extraction socket.
Methods The study was conducted in 10 patients visiting the outpatient Department of Oral & Maxillofacial Surgery, requiring extraction of bilateral mandibular third molars. Following extraction, autologous Platelet Rich Plasma (PRP) was placed in one extraction socket, the other socket was studied as the control site with no PRP. The patients were assessed for postoperative pain, soft tissue healing, bone blending and trabecular formation. Radiological assessment of the extraction site was done for a period of 4 months to evaluate the change in bone density.
Results Pain was less in the study site compared to control site, soft tissue healing was better in study site. Evaluation for bone blending and trabecular bone formation started earlier in PRP site compared to control, non PRP site. The evaluation of bone density by radiological assessment showed the grey level values calculated after 4 months at the PRP site were comparatively higher than the average baseline value of bone density at extraction site in control site.
Conclusion The study showed that autologous PRP is biocompatible and has significantly improved soft tissue healing, bone regeneration and increase in bone density in extraction sockets. However a more elaborate study with a larger number of clinical cases is essential to be more conclusive regarding its efficacy.

Keywords Platelet rich plasma · Mandibular third molar extract socket · Pain · Soft tissue healing · Bone trabeculae · Bone density

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Introduction

Oral implants, maxillofacial reconstruction, regenerative periodontal procedures are highly dependent on successful bone regeneration. Bone regenerative techniques including graft material, protein and barrier membranes are often used to improve bone quality before or during these treatment. Many studies both invitro and invivo have shown the effectiveness of growth factors that can enhance cell proliferation, differentiation, chemotaxis, and extracellular matrix synthesis involved in healing of tissues. Despite their potential usefulness, animal derived or genetically engineered growth factors are currently not available for regenerative therapies because their safety has not yet been completely confirmed.

Platelet-Rich Plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. Because it is a concentration of platelets, it is also a concentration of the 7 fundamental protein growth factors proved to be actively secreted by platelets to initiate wound healing. These growth factors include 3 isomers of platelet-derived growth factors (PDGFαα, PDGFββ, and PDGFαβ), 2 of the numerous transforming growth factors-β (TGFβ1 and TGFβ2), vascular endothelial growth factor, and epithelial growth factor. All these growth factors have been documented to exist in platelets. As these concentrated platelets are suspended in a small volume of plasma, PRP is more than just a platelets concentrate, it also contains the 3 proteins in blood known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue, and epithelial migration. These, cell adhesion molecules are fibrin, fibronectin and vitronectin [1]. The aim of this study was to evaluate the efficacy of autologous platelet- rich plasma in regeneration of bone and to assess clinical compatibility of the material in mandibular third molar extraction socket.

Materials and methods

The study was undertaken at the Department of Oral and Maxillofacial Surgery, after obtaining ethical committee clearance. This study involved both male and female patients, who were referred to the department of oral and maxillofacial surgery for removal of mandibular 3rd molar.

Inclusion criteria

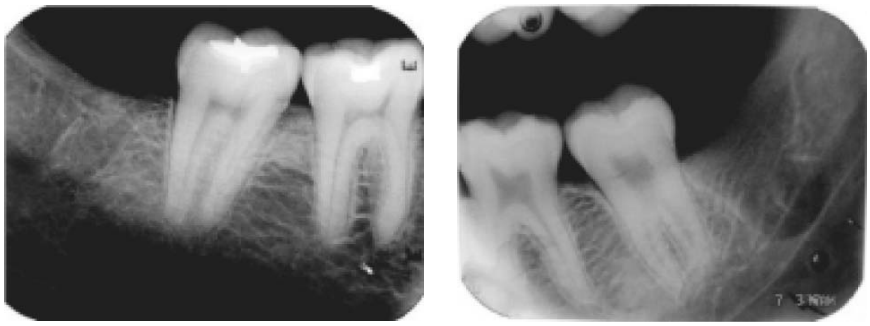
1. Patients aged between 18–45 years.
2. Patients who required bilateral mandibular 3rd molars extractions
3. ASA grade 1 patient
4. Patients who are non-smokers and non-alcoholics.

After obtaining the complete history, patients were examined clinically and were explained about the procedure, its complications and the follow-up period involved in the study. The patients who were willing were enrolled for the study. Informed consent was taken, study sample included 10 patients requiring bilateral mandibular 3rd molars extraction, all patients underwent bilateral removal of 3rd molars and autologous. PRP of that patients was prepared by taking 10ml of blood and centrifuged in laboratory and PRP was separated from blood. It was then placed in the extraction sockets on one side and other side no PRP is placed. Both the sockets are closed primarily by suture.

All patients were recalled on day 1, day 3, day 7, 2 months, 3 months, and 4 months, postoperatively for follow-up study. Clinical evaluation included assessment of pain, soft tissue healing, pain was evaluated using the Visual Analogue Scale. Evaluation of soft tissue healing by Landry and Turnbull [2,3], IOPA radiographs were taken preoperatively, and at 8 week, 12 week, 16 weeks postoperatively to assess and compare radiographic bone densities between PRP sites and non-PRP sites. All the data was entered into a MS Excel sheet and statistically analysed with the help of students t-test and ANOVA test.

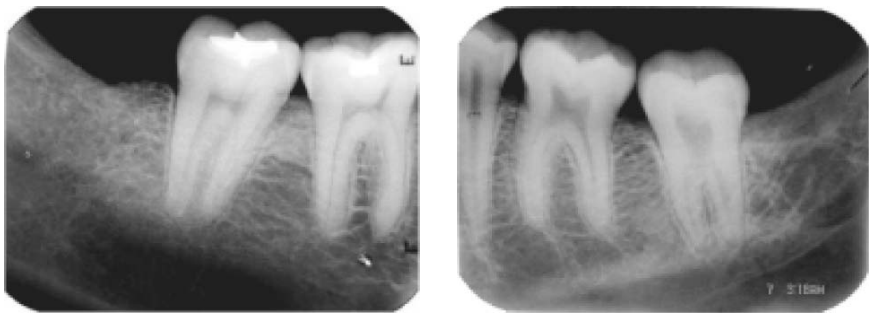
Results

Following completion of clinical study on the patients, the measurements and data taken from all the patients were tabulated for statistical studies. After analysis of the data



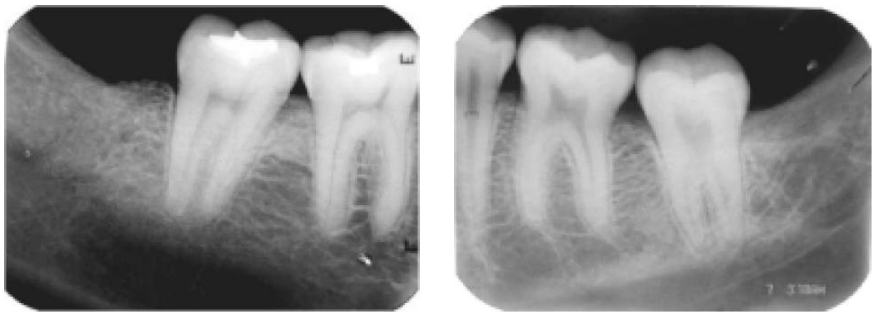
Right side (study) Left side (control)

Fig. 1 Radiograph (IOPA) at 8 weeks postoperatively



Right side (study) Left side (control)

Fig. 2 At 12 weeks postoperatively



Right side (study) Left side (control)

Fig. 3 16 weeks postoperatively

the following observations were made. There were 5(50%) male subjects and 5(50%) female subjects who participated in the study. The patients who had participated in the study were in the age of 18 years to 45 years, with a mean age of 27 years.

Results of clinical assessment

Assessment of pain by Visual Analogue Scale on the first day showed mean pain score of 3.9 in study site and 4.1 in control site, on 3rd day mean pain score was 1.2 in study site and 1.5 in control site, on 7th day score was 0 in both study and control site, though pain was less in study site compared to control site, there was no statistically significant difference between study and control group at 1st day, 3rd day and 7th day. By doing Bonferroni multiple comparison test there was no significant difference between 1st day to 3rd day (p value:.005),3rd day to 7th day(p value: .035), and 1st day to 7th day (p value: .0005).

Assessment of the healing index of soft tissue

Assessment of soft tissue healing by healing index by Landry, Turnbell and Howley showed mean score on 1st day of 3.5 in study site, 2.9 in control site. On 3rd day 4.2 in study site, 3.4 in control site on 7th day mean score of 4.8 in study site and 4.3 in control site, by doing repeated ANOVA measure test for study and control group healing was better for study site compared

Chart 1 Assessment of pain using VAS

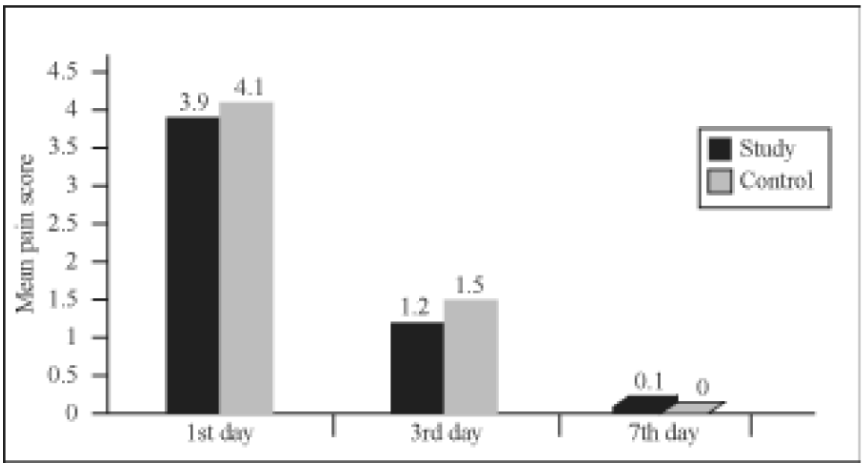


Chart 2 Assessment of healing index postoperative

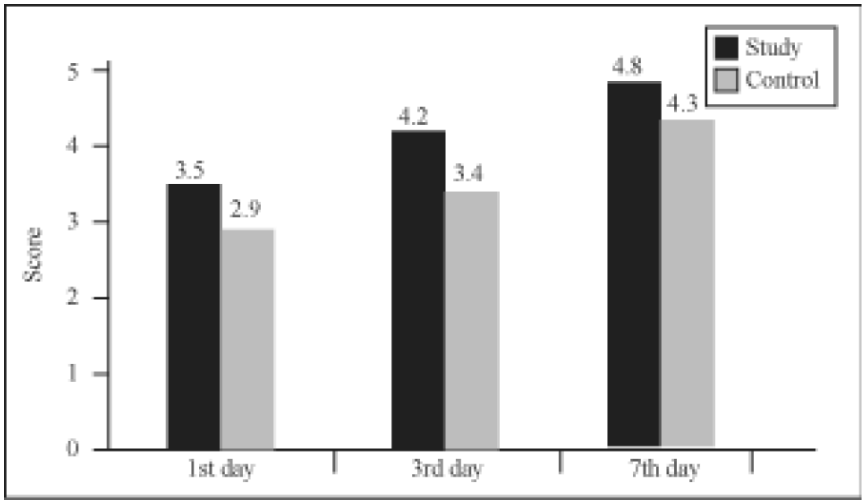
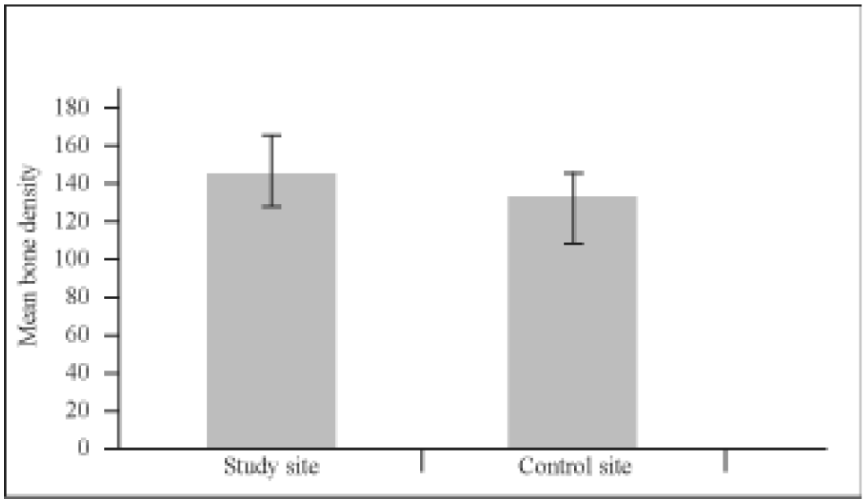


Chart 3 Assessment of bone density on postoperative radiographs



to control site between 3rd day to 7th day (p value for 3rd day - 0.022 and 0.015 for 7th day), there was significant difference between the study and control sites in all the 10 patients.

Radiographic assessment

Radiographic assessment at 8 weeks for bone margins blending seen in 7 patients in both study and control site. Macnamer chi-square test shows p value of 1.0. Trabecular bone formation seen in 4 patients at study site but absent in all the 10 patients at control site.

At 12 weeks: Blending of bone margins was seen in all the 9 patients in both study and control sites except in 1 patient it was seen in study site but absent in control site of that patient. Trabecular bone formation seen in 9 patients in study site but only in 5 patients in control site, (p value 0.063), there was significant difference between the PRP site and Non PRP site.

At 16 weeks: Blending of bone was seen in all 10 patients in both PRP (study) site and Non PRP (control) site. Trabecular bone formation also seen in all 10 patients in both the sites. Assessment of bone density (gray level value) at 16 weeks shows, the average gray scale value for PRP (study) site (146.9) was comparatively higher than Non PRP (control) site (123).

Discussion

During the last decade, there have been several in vivo animal studies, which have used biological mediators such as polypeptide growth factors to expedite soft tissue and bony healing. TGF b1 and b2 have shown to inhibit bone resorption, osteoclast formation and activity, as well as to trigger rapid maturation of collagen in early wounds. PGDF increases the population of wound-healing cells and recruits other angiogenic factors to the wound site. It is therefore a reasonable hypothesis that increasing the concentration of platelets in bone defects may lead to improved, faster healing and stimulate new bone formation [4].

Using PRP involves taking a sample of a patient's blood preoperatively, concentrating autologous platelets and applying the resultant gel to the surgical site. This technique produces a blood clot that has nearly a reverse ratio of red blood cells and platelets compared with a natural clot. Surgical sites enhanced with PRP have been shown to heal at two to three times that of normal surgical sites. Thus, PRP can be a great adjunct to many surgical procedures [5,6,7].

There are various methods for preparing PRP. Techniques vary from using 10 cc of patient's blood and spinning it in a clinical centrifuge, to using a unit (400–500 cc) of blood that is put through a cell separator that sequesters and concentrates the platelets [8,9]. In our study PRP was prepared from 10 cc of patient's blood and centrifuging the blood twice in a clinical centrifuge as advocated by various authors [5,6,7].

The PRP is activated to form PRP gel thus causing degranulation of α-granules present in the platelets and releasing the growth factors. The various agents for the activation reported in literature include $CaCl_2$ alone, $CaCl_2$ plus bovine thrombin, human thrombin, autologous bone or whole blood which contains thrombin. Bovine thrombin was not utilized in our study since its use was associated with the development of antibodies to clotting factors V, XI, and thrombin, results in the risk of life threatening coagulopathies [10].

In our technique $CaCl_2$ alone was mixed with PRP to form an autologous platelet gel. This platelet gel was free of eliciting any antigen-antibody reaction as it was prepared from patients own blood.

Landesberg et al. (2000) [11] observed that the use of Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant caused fragmentation of platelets. Our study used citrate phosphate dextrose due to its compatibility with platelet membranes.

Juan et al. (2000) [12] elaborated the characteristics of PRP:

- It provides adhesiveness & tensile strength for clot stabilization
- It is biologically acceptable
- It contains important growth factors such as PDGF & TGF-B
- It has hemostatic properties & Promotes Angiogenesis
- It improves wound healing
- It is highly osteoconductive

Our results with regard to the enhanced soft tissue healing and increased rate of bone formation may be due to the above mentioned advantages of PRP. No graft material was added to PRP in this study, as in other studies like Marx et al. (1998) [9], Anitua (1999) [10], Kassolis et al. [13]. It is assumed that the combination of bone grafts with PRP. We found better soft tissue healing of extraction sockets with PRP as compared to the Non-PRP sockets. Our finding is supported by the Deepti Simon et al. [14], who in their study reported that soft tissue healing was significantly better in the cases where the extraction sockets were treated with PRP.

Radiographic evaluation results at 8 weeks shows not much difference in bone blending between study and control site but at 12 weeks trabecular bone formation seen in 9 patients in study site but only in 5 patients in control site there was significant difference between the PRP site and Non PRP site. At 16 weeks on assessment of bone density (gray level value) shows [15], the average gray scale value for PRP site 146.9 was comparatively higher than Non PRP site at 123.

Conclusion

In this study autologous PRP was used as an adjunct to promote wound healing and osseous regeneration in human mandibular third molar extraction sites. It clearly indicates a definite improvement in the soft tissue healing and faster regeneration of bone after third molar surgery in cases treated with PRP as compared to the control group postoperatively. This improvement in the wound healing, decrease in pain, and increase in the bone density signifies and highlights the use of PRP, certainly as a valid method in inducing and accelerating soft and hard tissue regeneration. Moreover the preparation of PRP by collecting blood in the immediate preoperative period avoids a time consuming visit to blood bank for the patient. An added benefit of PRP noted in the present study is its ability to form a biologic gel that provided clot stability and function as an adhesive. The procedure of PRP preparation is simple, cost effective and has demonstrated good results.

The present study was done with a follow up of 4 months, further clinical trials with longer duration follow up with larger sample size should be done to get more affirmative and conclusive results.

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