Efficacy of platelet rich plasma in bone regeneration after surgical removal of impacted bilateral mandibular third molars: pilot study

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Received: 8 October 2009 / Accepted: 2 November 2009

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Abstract

Purpose This study was done to assess the efficacy of platelet rich plasma (PRP) in bone regeneration.

Materials and methods 20 patients having bilateral mandibular third molar impaction with similar angulations were included in the present study. The extraction socket was packed with PRP on one side and the other side was sutured without PRP. The bone density of both extraction sockets were evaluated radiographically using gray level histogram and compared periodically. Postoperative pain and edema were also assessed.

Results The result of the study shows rapid bone regeneration in the extraction socket treated with PRP when compared with the socket without PRP. Also there was less postoperative discomfort on the PRP treated side.

Conclusion Within the limits of the present study it can be concluded that usage of PRP expedites bone regeneration, and reduces postoperative complications.

Keywords Platelet rich plasma · Growth factors · Third molar · Bone regeneration

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Introduction

Platelet Rich Plasma (PRP) is an autologous concentrate of platelets suspended in plasma [1,2,3]. It is a proven source of growth factors like Platelet Derived Growth Factors (PDGF) and Transforming Growth Factor (TGF)- β 1 and 2; which is obtained by sequestering and concentrating platelets by gradient density centrifugation [3,4,5]. While combining with calcium chloride and thrombin, PRP releases these growth factors. Platelet rich plasma gel also contains a native concentration of fibrinogen. As a result, it permits stabilized coagulation of blood thereby favoring regeneration of the osseous defects particularly in the early stage [3,4,5].

Soft tissue healing is also substantially improved through the application of PRP, by increasing collagen content, promoting angiogenesis and increasing early wound strength [1]. The growth factors found in

PRP regulate key cellular processes, such as chemotaxis, cell differentiation and metabolism [4].

This study was carried out to evaluate the efficacy of PRP on bone regeneration after surgical removal of mandibular third molars. The results were evaluated clinically and radiographically.

Patients and methods

The present study was undertaken in the Department of Oral and Maxillofacial Surgery, at our institute with due permission of the ethical committee. A total of 20 patients; both male and female aged between 18 to 30 years, who had bilateral impacted mandibular third molars with similar angulation were selected for this study with informed consent. Selected patients were screened for any infection in the region of lower third molars, any systemic diseases, oral habits, allergy or

hypersensitivity to local anaesthetics. Platelet count checked to ascertain its normalcy.

Preoperative preparation of platelet rich plasma

In each of two vacutainers of 6ml capacity (Becton & Dickinson) containing CPDA (Citrate Phosphate Dextrose Adenin) anticoagulant solution 5ml of blood was collected from antecubital region. Both the tubes were placed in centrifuge machine (Digital Centrifugation Machine REMI Motors Ltd.) and were counter balanced. The first centrifuge cycle was done at 1200 rpm for 10 minutes. The result was separation of the whole blood into a lower red blood cell region and upper straw coloured plasma (Fig. 1). This plasma contains relatively low concentration of platelets (platelet poor plasma) in the uppermost region and higher concentration of platelet in the boundary

Fig. 1 Blood after first centrifugation

Fig. 2 Withdrawal of top layer

Fig. 3 After second centrifugation

Fig. 4 Concentrated PRP

Fig. 5 Addition of CaCl_2 and autologous thrombin to cPRP

Fig. 6 Platelet rich plasma gel

Fig. 7 Placement of PRP gel in the extraction socket of third molar

Fig. 8 Gray level histogram value measurement using Adobe Photoshop

layer often called as 'Buffy coat'. The tubes were placed in a rack with their top uncovered. An equal number of 6ml tubes without anticoagulant solution were also placed in the rack with their top uncovered.

A spinal needle (18 gauge and 78 mm long) was attached to 5ml syringe to withdraw straw coloured plasma from the tube. The drawing of plasma was stopped when the RBC layer was reached (Fig. 2). The straw coloured plasma was then expressed into the tube without anticoagulant solution. This process was done gently so as not to disturb the platelets. Same procedure was performed

with the other tubes. The tubes with straw coloured plasma were centrifuged again at 2000 rpm for 10 minutes.

The tubes were placed in the rack with their top uncovered. Now the contents of the tube consist of upper layer of clear supernatant serum containing fibrinogen and very low concentration of platelets. The bottom layer often red tinged consists of highly concentrated platelets (Fig. 3).

The upper 2/3 of this liquid was withdrawn with same spinal needle attached to 5ml syringe leaving behind 1/3 of the serum with concentrated platelets.

This remaining content of the tube was thoroughly mixed to form red coloured concentrated platelet rich plasma (cPRP) (Fig. 4).

Autologous thrombin was recovered from a portion of cPRP briefly 2ml of cPRP was treated with 60 microlitre of citrate inhibitor sterile 10% calcium chloride solution, allowed to coagulate and then squeezed to release the platelet derived thrombin and collected in serum rich plasma.

Autologous thrombin rich plasma was mixed with cPRP in the 1:4 ratio and

Fig. 9 Comparison of the IOPA radiographs
A&B: Immediate postoperative IOPA radiographs
of control and study
C&D: One month postoperative IOPA
radiographs of control & study
E&F: Three months postoperative IOPA
radiographs of control & study
G&H: Six months postoperative IOPA
radiographs of control & study

allowed to coagulate (Fig. 5). PRP gel was formed within 5 to 10 minutes (Fig. 6). All the impacted teeth were removed surgically by a single operator under local anesthesia. PRP gel was placed in the experimental extraction site before closure of the wound (Fig. 7). The control extraction site was closed without placing the PRP gel.

All the patients were evaluated for pain and swelling on 2nd and 7th postoperative day. Visual Analogue Scale (VAS) was used as a subjective method to evaluate the pain [6]. Swelling was measured preoperatively on 2nd and 7th postoperative day and recorded [7]. IOPA radiographs and OPG were taken as a standard protocol on 2nd postoperative day as well as on 1st, 3rd and 6th month postoperatively.

For bone density measurement all the IOPA images were scanned and digitalized. The mean grey level histogram values of the scanned IOPA images of the extraction sockets were obtained through Adobe Photoshop 7.0 software (Fig. 8).

Results

The results were evaluated based on clinical observation, radiographic analysis and

Graph 1 Comparison of mean values of gray level histogram in control and study group

Graph 2 Comparison of difference of mean values of gray level histogram from postoperative to 1 month, 3 months and 6 months in control and study group

Graph 3 Mean percentage (%) increase in gray level histogram value from postoperative to 1 month, 3 months and 6 months in control and study group

densitometric analysis by gray level histogram. All the results were calculated using the mean value and standard deviation for each of the parameters considered and checked for

statistical significance using unpaired t-test. We found that the difference between mean values of gray level histogram was significant and highly significant at 3rd month and 6th month

Graph 4 Comparison of mean % of swelling in control and study group **Graph 5** Comparison of mean values of VAS for pain in control and study group

postoperatively in control and study group (Table 1 and Graph 1).

We found that there was significant difference between mean values % of swelling in control and study group at 2nd and 7th postoperative day suggesting that percentage of facial swelling in control group is higher than that of study group (Table 4 and Graph 4). Also there was no significant difference between mean values of VAS for pain in control and study group (Table 5 and Graph 4).

Discussion

Surgeons are continuously searching for ways to improve bone healing and success of bone grafting with either autogenous bone or other bone substitutes. One of the most recent and innovative technique which has come up is the use of PRP [8].

The theory behind PRP is compelling. It is now known that platelets have many functions beyond that of simple

haemostasis. Platelets contain important growth factors, that when secreted, are responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular ingrowth, and, inducing cell differentiation. These are crucial steps in early wound healing [8].

Using the concept that increasing the concentration of platelets at a wound site may promote more rapid and better healing, it seems very logical that increasing the concentration of platelets in the bone graft and therefore increasing the concentration of growth factor may lead to more rapid and denser bone regeneration [8].

Whitman et al. [9] who introduced PRP to Oral and Maxillofacial Surgical community, used it as fibrin glue for faster soft tissue healing. It was thought that through activation of the platelets within the gel and the resultant release of the growth factors, enhanced wound healing should be expected. Robert Marx et al. in 1998, who popularized PRP in Oral and

Maxillofacial surgery after the publication of their landmark article concluding that combining PRP with autogenous bone in mandibular continuity defects resulted in significantly faster radiographic maturation and histomorphometrically denser bone regenerate [3].

A number of authors in their invivo [10] studies have highlighted the importance of growth factors found in PRP. The positive effects of PRP on tissue regeneration, a growing number of human clinical studies have detailed the use of growth factors in reconstructive oral and maxillofacial surgery [3,11], periodontology [12,13,14], implantology [15,16], pre-prosthetic surgeries [14,16], sinus grafting [16], distraction osteogenesis [17,18] alveolar bone grafting [19], and plastic surgery [20].

There are various methods of PRP preparation from autologous blood. Techniques vary from using 10cc of blood in a clinical laboratory centrifuge [15,20,21,22,23] to using a unit (250–450cc) of whole blood that is put in the cell separator; they sequester and concentrate the platelets [2,3,14]. At a minimum, any procedure developed to concentrate platelets should be able to show a sufficiently high concentrate of platelets to affect a clinical outcome and demonstrate high percentage of growth factor in the resulting concentrate, as well as a variable platelet population [3,21]. So in our study PRP was prepared from 10cc of patient's blood and centrifuged twice in a clinical tabletop digital centrifuge as advocated by Aron Gonshor [21], Regina Landesberg et al. [24], Rick C Tsay et al. [25].

To date, there are no clear dose response curves of platelet concentration and bone enhancement; although an increase of 3 to 5 times the whole blood baseline level is considered a clinical benchmark given by Marx et al. [3,4]. The procedure we follow meets the benchmark criteria. The platelet count in the PRP used in our study was 4–5 times the base line value and was in tandem with the study done by Marx et al. [3,4,25,26].

PRP when activated to form gel causes degranulation of α granules in the platelets releasing number of known growth factors. PRP can be activated with calcium chloride alone [15], calcium chloride along with bovine thrombin [4,25,26], human thrombin, autologous bone or autologous blood containing thrombin [21], TRAP (Thrombin Receptor Activator Peptide) [24,25] or ITA gelling agent [24].

In our study, autologous thrombin was prepared and mixed with PRP to form PRP gel [14,18,27]. This platelet gel was free

Table 1 Comparison of mean values of gray level histogram in control and study group: (unpaired 't' test)

	Control (n=20) Mean $\pm$ SD	Study (n=20) Mean $\pm$ SD	't' value	'p' value	Result
Postoperative	48.62 $\pm$ 8.12	49.22 $\pm$ 7.74	0.24	p>0.05	Not significant
1 month	71.83 $\pm$ 7.23	74.64 $\pm$ 6.94	1.26	p>0.05	Not significant
3 months	85.88 $\pm$ 7.23	90.76 $\pm$ 7.01	2.17	p<0.05	Significant
6 months	94.43 $\pm$ 7.15	100.53 $\pm$ 6.93	2.73	p<0.01	Highly significant

Table 2 Comparison of difference of mean values of gray level histogram from postoperative to 1 month, 3 months and 6 months in control and study group: (unpaired 't' test)

	Control (n=20) Mean $\pm$ SD	Study (n=20) Mean $\pm$ SD	't' value	'p' value	Result
Postoperative to 1 month	23.21 $\pm$ 8.84	25.39 $\pm$ 8.12	0.82	p>0.05	Not significant
Postoperative to 3 months	37.26 $\pm$ 6.73	41.54 $\pm$ 6.88	1.99	p<0.05	Significant
Postoperative to 6 months	45.81 $\pm$ 6.09	59.31 $\pm$ 7.14	6.43	p<0.01	Highly significant

Table 3 Comparison of percentage change from postoperative to 1 month, 3 months and 6 months of gray level histogram in control and study group

	Control (n=20)	Study (n=20)
Postoperative to 1 month	47.74%	51.64%
Postoperative to 3 months	76.63%	84.40%
Postoperative to 6 months	94.2%	104.25%

Table 4 Comparison of mean values of % of swelling in control and study group

	Control (n=20) Mean $\pm$ SD	Study (n=20) Mean $\pm$ SD	't' value	'p' value	Result
Preoperative	0 $\pm$ 0	0 $\pm$ 0	0	p>0.05	Not significant
2nd postoperative	4.45 $\pm$ 2.64	2.81 $\pm$ 1.58	8.03	p<0.05	Significant
7th postoperative	0.14 $\pm$ 0.3	0.046 $\pm$ 0.20	1.97	p<0.05	Significant

Table 5 Comparison of mean values of VAS for pain in control and study group (Unpaired 't' test)

Days	Control (n=20) Mean $\pm$ SD	Study n=20 Mean $\pm$ SD	't' value	'p' value	Result
1	2.1 $\pm$ 0.62	1.8 $\pm$ 0.75	1.38	p>0.05	Not significant
2	1.8 $\pm$ 0.6	1.4 $\pm$ 0.58	1.64	p>0.05	Not significant
3	1.05 $\pm$ 0.58	0.65 $\pm$ 0.65	1.59	p>0.05	Not significant
4	0.3 $\pm$ 0.46	0.25 $\pm$ 0.43	1.43	p>0.05	Not significant
5	0.1 $\pm$ 0.3	0.05 $\pm$ 0.22	1.87	p>0.05	Not significant
6	0 $\pm$ 0	0 $\pm$ 0	0	p>0.05	Not significant
7	0 $\pm$ 0	0 $\pm$ 0	0	p>0.05	Not significant

of any antigen antibody reaction as it was prepared from patients own blood.

Regina Landesberg [2] and Robert Marx [3] stated that use of EDTA is potentially more harmful than citrate. Although EDTA gave greater yields of platelets, they appeared damaged by presence of EDTA [2].

So we used CPDA anticoagulant solution in our study as it maintains or preserves platelet membrane integrity [22].

The positive effects of PRP as reported in literature are [1,3,28,29]:

- 'Jump starts' the cascade of osteogenesis in bone graft.

- Improves trabecular bone density.
- Provides earlier availability of growth factors and BMP.
- Promotes early consolidation of the graft.
- Hastens the mineralization of the graft.

In the present study PRP was placed in the third molar extraction sockets. We found that there was good soft tissue healing response in PRP treated sites as compared to the other site.

The density of the bone formed in the extraction socket was calculated by measuring the gray level histogram values of the digitalized IOPA radiograph images. This method to assess the bone density was based on the suggestion given by Damante et al. who measured the radiographic density to assess the resolution of the bone cyst and recommended the technique as an useful tool [4].

In the present study there was significant difference in the mean values of gray level histogram at 3 rd month and 6 th month postoperatively but, it was not significant immediately after extraction and at the 1 st month postoperatively, as shown in table and bar diagrams I and II. We assessed the percentage of facial swelling in both study and control side according to the formula given by Mohammad Amin et al. [7]. The percentage of facial swelling was greater in the control side as compared to the study side at 48 hours postoperatively and on the 7th postoperative day.

Pain was also assessed with the help of VAS and it was found that the severity of pain was equal on both the study and control side. The results were not significant any time during day 1 to day 7. However the intensity of pain was higher on the day of the surgery and gradually decreased in both sides.

Our findings were supported by Deepti Simon et al. [22]. However in contrast to our study; the swelling was also similar on both control and study sides in their study.

Jerome Mancuso et al. also reported more dense radiographic bone healing, decrease in swelling postoperatively, better haemostasis, and lower level of pain on the third molar extraction site treated with PRP [30]. Similarly we also found dense radiographic bone healing (Fig. 9). Anitua reported improved epithelialization and bone density when PRP was placed in the extraction sockets [15].

Conclusion

The present study demonstrates that PRP expedites bone regeneration and has less postoperative complications as compared to the extraction socket that healed without

PRP. The limitation of the present study was that the postoperative follow up is a short duration (6 months) to arrive at a definitive conclusion on the efficacy of PRP in complete bone regeneration process but adequate enough to evaluate the effects of PRP in initiating and enhancing both hard and soft tissue healing. Long term follow up and large sample size is required along with histological study of the bone for assessment of the efficacy of PRP.

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Source of Support: Nil, **Conflict of interest:** None declared.