

# Periodic Research

## Role of PRP in Periapical Defects

### Abstract

This study was conducted to assess the efficacy of platelet rich plasma in periapical defects. 11 patients with periapical radicular cyst were selected for the study. After surgical enucleation of the cyst, PRP was filled in cystic cavity. Osseous regeneration was evaluated using IOPAR at end of 1, 3 and 6 months. At the end of 6 months, significant amount of blending of margins and formation of trabecular bone was observed. PRP was found to be effective in osseous regeneration of small cystic cavities.

**Keywords:** Radicular Cyst, Regeneration, Healing.

### Introduction

The past two decades have witnessed a change in the use of bone grafts in the human biologic system from mere fact of use and observation of the outcome, to a scientific dynamic endeavor. Various tissue engineering techniques<sup>1</sup> have come up recently. One such recent innovation is the preparation and use of platelet rich plasma (PRP), which is a concentrate of platelets.<sup>2,3</sup> It is now well known that platelets have many functions beyond that of hemostasis. 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 in-growth, and inducing cell differentiation. These polypeptide growth factors as well as other bioactive substances are released from platelets, which upon activation, play a pivotal role in initiating and sustaining wound healing<sup>4</sup> and tissue repair mechanism.

Our aim is to evaluate the efficacy of the platelet rich plasma in periapical defects, hence the study is planned to assess the Osseo regenerative capability and advantages of PRP using conventional intraoral Periapical radiographs.

### Review of Literature

Dean H. Whitman et al (1997),<sup>5</sup> reported the use of Platelet gel as an alternative to fibrin glue. This gel which contained a high concentration of platelets and a native concentration of fibrinogen is prepared in immediate preoperative period.

Christopher L. Strayhorn et al (1999),<sup>6</sup> conducted an in-vitro study to evaluate the effects of various growth factors i.e. PDGF, IGF, and BMPs alone or in combination on expression of Osteoblast - associated genes in osteoblast differentiation. The study showed that all these factors in combination enhance the biologic activity of osteoblasts.

Yong-Moo Lee, et al (2000),<sup>7</sup> conducted a study to evaluate the bone regenerative effect of platelet derived growth factor- $\beta\beta$  [PDGF- $\beta\beta$ ] when delivered with a Chitosan/ Tricalcium Phosphate [TCP] sponge carrier in a rat calvarial defect model. The histological examination showed that the Chitosan/ TCP sponge carrier promoted osseous healing of the Rat calvarial defects as compared to controls. The results also showed that PDGF-  $\beta\beta$  further enhanced bone regeneration.

Robert E. Marx (2000),<sup>8</sup> stated that use of Ethylene Di amine- Tetra-acetic Acid (EDTA) as anticoagulant is not recommended because it fragments platelets. Acid citrate dextrose (ACD) was therefore preferred, as the anticoagulant of choice since it preserves platelet membrane integrity.

Richard Shanaman, R. Filstein, Michael J. Danesh Meyer (2001),<sup>9</sup> conducted a case series on localized ridge augmentation using guided bone regeneration (GBR) and PRP.

M. Schiellphake (2002),<sup>10</sup> published a review based on the results of 231 references to survey the available information on the potential of bone growth factors in reconstruction of the maxillofacial area.

Antonio Della Valle et al (2003),<sup>11</sup> conducted a study in which 40 patients on anti-coagulant drugs underwent extractions and platelet rich plasma gel was put into residual alveolar bone defects. The results showed



**Swati Sharma**  
Senior Lecturer,  
Dept. of Orthodontist,  
Eklavya Dental College,  
Kotputli, Rajasthan, India

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that 5% reported hemorrhagic complications, 40% had mild bleeding the remaining 55% presented with adequate hemostasis.

Tolga Fikret Tözüm, Burak Demiralp (2003),<sup>12</sup> in an article outline the specific effects of growth factors present in PRP, both in vitro and in vivo, on periodontal wound healing. The authors concluded that PRP is a new application of tissue engineering and a developing area for clinicians and researchers.

Burak Demiralp et al (2004),<sup>13</sup> was the first to report the use of platelet rich plasma in Periapical surgical therapy. In his case report a Periapical lesion was present in the apical region of both maxillary central incisors which were non vital. Root canal treatment was done in relation to both the teeth, followed by surgical curettage of the area 6 months after the root canal treatment.

Earl G. Freymiller, Tara L. Aghaloo (2004),<sup>14</sup> in a review article based on 34 references concluded that all clinicians involved in bone grafting have high hopes that PRP would eventually prove to be great benefit in bone graft healing.

O. Kilian et al (2004),<sup>15</sup> conducted a study to investigate the effects of enriched platelet derived growth factors on proliferation & migration of human endothelial and mesenchymal stem cells on osteogenic differentiation of stem cells. In this study PRP was prepared with 500 ml of blood and was activated with thrombin and calcium gluconate for aggregation and degranulation of the platelets.

Kim TH et al (2014),<sup>26</sup> compared PRP with PRF (Platelet rich fibrin) and CGF (Concentrated growth factor) and found significantly increased bone formation at the end of 6<sup>th</sup> week in all the cases.

Hsu CW et al (2016),<sup>24</sup> recommended use of bone replacement analogue along with membranes in case of large lesions.

## Materials and Methods

In this study 11 cases who reported to the department of oral and maxillofacial surgery presenting with the Periapical defects were selected for the study.

Inclusion criteria-

1. Patients above 15 years of age were selected for the study.

## Exclusion Criteria

1. Patients suffering from any systemic disorders
2. Patients with blood dyscrasiasis.
3. Patients on antiplatelet/ anticoagulant therapy.
4. Patients with history of recent myocardial infarction
5. Pregnant women.

A detailed case history of the patient was obtained. An informed consent was obtained from the patients regarding the surgery as well as the use of platelet rich plasma and hydroxyapatite.

In this study, all patients were diagnosed as radicular cyst. The offending teeth was saved conservatively by performing root canal therapy among 6 of the patients and in remaining 4 patients offending tooth was extracted due to poor prognosis, followed by which enucleation of the cystic lining and placement of platelet rich plasma was done.

## Platelet Rich Plasma: An Insight

Platelet Rich Plasma (PRP) is an autologous concentration of human platelets in a small volume of Plasma.

PRP has been shown to contain various growth factors<sup>2, 10, 17, 18, 19</sup>, including platelet derived growth factor (PDGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), Insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), and Platelet derived epidermal growth factor (PDEGF).

## Preparation of Platelet rich plasma<sup>10</sup>

1. 20 - 100 ml blood was drawn from the patient by venepuncture at the antecubital fossa depending on the size of the lesion.
2. This blood was transferred into a pre-sterilized vacutainer centrifugation tube (GUANGZHOU IMPROVE MEDICAL INSTRUMENTS CO, LTD) containing 0.109M of anticoagulant Trisodium Citrate (blue colour cap).
3. This blood sample was then centrifuged at 1300 rpm for 10 minutes to be separated into a lower red blood cell region (RBC) region and upper straw coloured plasma region. This plasma contains low concentration of platelets (platelet poor plasma; PPP) in the uppermost region and a higher platelet concentration in the boundary layer, often called —Buffy coat
4. 5 ml syringe with 0.6x25 mm needle was used to aspirate the straw coloured plasma (PPP), Buffy coat and 1 to 2 mm of the top portion of the RBC layer.
5. It was then transferred to another pre-sterilized vacutainer centrifugation tube without any anticoagulant (red colour cap) and centrifuged at 2000 rpm for 10 minutes to separate an upper portion of clear yellow supernatant serum, containing fibrinogen and a very low concentration of platelets; the bottom layer, often red tinged consists of highly concentrated PRP.
6. The top serum layer containing fibrinogen and low concentration of platelets is thrown off, leaving 1.4 ml- 7 ml of serum with PRP in the tube, so that it can be kept dissolved in it, depending on the preparation.
7. The contents of the tube were then expressed into a sterile container.
8. 0.5 ml of 10% calcium gluconate<sup>17</sup> was added to the PRP to get a gel like consistency.

In our technique calcium gluconate 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.

Periapical surgery was carried out under local anaesthesia, PRP was prepared and activated with calcium gluconate and was placed in the base and wall of the defect. Wound is closed primarily with 3-0 silk sutures. Follow up radiographs were taken at 1<sup>st</sup> month, 3<sup>rd</sup> month and 6<sup>th</sup> month interval.

## Evaluation of Osseous Regeneration

Osseous regeneration was evaluated using conventional intraoral periapical radiographs. The criteria for evaluating bone regeneration were:

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## 1. Blending of margins

- a) Blending of margins: (+)
- b) Persistence of margins: (-)

## 2. Trabecular bone formation

- a) Presence of trabecular bone: (+)
- b) Absence of trabecular bone: (-)

### Results

This study was aimed at evaluating the efficacy of platelet rich plasma in the healing of the periapical defects using conventional intra oral Periapical radiograph.

This study included eleven patients who required surgical enucleation of the defect in the Periapical region. Out of the eleven patients, nine were male and two were female. One patient did not come for the review. The age of the subjects ranged from 21 to 38 years. Periapical surgery was carried out and the defect was grafted with PRP. Osseous

regeneration was evaluated using routine intra oral periapical radiograph (IOPAR), which were taken at the end of first month, third month and sixth month post-operatively. The results of are tabulated in the table 1.

At the end of 1<sup>st</sup> month, there was no blending of margins and no trabecular bone formation.

At the end of 3<sup>rd</sup> month, 6 of them showed good amount of blending of margins and 4 of them had less amount of blending of margins. Trabecular bone formation was not clearly but was evident in 2 subjects

At the end of 6<sup>th</sup> month, there was significantly perfect blending of margins in all the subjects. Trabecular formation was significantly evident.

**Table-1**  
**Evaluation of Osseous Regeneration**

Criteria	Blending of margins			Presence of Trabecular Bone		
	1st	3rd	6th	1st	3rd	6th
Sr. No						
1.	-	+	+	-	-	+
2.	-	+	+	-	-	+
3.	-	+	+	-	+	+
4.	-	+	+	-	-	+
5.	-	+	+	-	-	+
6.	-	+	+	-	-	+
7.	-	+	+	-	+	+
8.	-	+	+	-	-	+
9.	-	+	+	-	-	+
10.	-	+	+	-	-	+

### Discussion

Large lesions may need to be treated with endodontic therapy or extraction combined with a biopsy or enucleation or marsupialisation procedures.

The radicular cyst constitutes 70% of all the jaw cysts.<sup>20</sup> The exact mechanism by which Periapical lesions are formed is not clearly understood, but it seems that products released by microorganisms and necrotic pulp may initiate the process of the inflammatory reaction. Periapical granulomas respond well to non surgical endodontic treatment, whereas radicular cysts are generally considered to require surgery.<sup>21</sup>

Bone healing after surgical procedure usually occurs by osteogenesis, by incorporating graft material into the surgical area triad of mechanism occurs- osteogenesis, osteoinduction and osteoconduction. Successful incorporation of graft material heals quicker, revascularizes and eventually assumes the desired form required for secondary procedures.

Apart from using bone grafts, various tissue engineering techniques have come up recently. One such recent innovation is the preparation and use of platelet rich plasma (PRP), which is a concentration of platelets and growth factors found in platelets. Using the concept that if a few are good, then a lot may be better and therefore increasing the concentration of platelets at a wound site may promote more rapid healing. Surgical sites enhanced with PRP have been shown to heal 1.62 times to 2.16 times more than that

of normal surgical sites without PRP.<sup>2</sup> PRP can be a great adjunct in various surgical fields, including oral and maxillofacial surgery<sup>2, 3, 5, 17, 18, 22</sup>, cardiology<sup>23</sup>, plastic surgery<sup>4</sup>, trauma to enhance wound healing and regeneration. PRP is a component of blood in which the platelets are concentrated in a limited volume of plasma<sup>2, 3, and 12</sup>. Therefore the study was planned to evaluate the efficacy of PRP along with alloplastic material in healing of the Periapical defects using conventional periapical radiograph. The study included 11 patients requiring surgical enucleation of the Periapical defects.

PRP has been shown to contain various growth factors<sup>2, 19</sup> including platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), Insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), and Platelet derived epidermal growth factor (PDEGF). These polypeptide growth factors as well as other bioactive substances are released from platelets upon activation and play a pivotal role in initiating and sustaining wound healing and tissue repair mechanism.<sup>2</sup> PRP decreases the frequency of Intraoperative and postoperative bleeding<sup>13</sup> and facilitates more rapid healing by promoting rapid vascularisation of the healing tissue by delivering growth factors.

The first clinical dental results with PRP were reported by Marx et al<sup>2</sup> in 1998. Their data strongly suggested that adding PRP to bone grafts accelerated the rate and degree of bone formation. Burak Demiralp et al<sup>13</sup> were the first to use PRP for

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Periapical surgical therapy where they have used the combination of PRP with tricalcium phosphate, the result was satisfactory and tricalcium phosphate was totally resorbed and replaced by new bone by 12 months.

PRP along with growth factors also secretes thrombospondins (TSPs) <sup>24</sup>. TSPs are large extracellular matrix proteins that influence the adhesion, migration, proliferation, survival and differentiation of a variety of cell types. TSPs -1 inhibits adhesion, proliferation, and tube formation of endothelial cells in culture and it has been found to block neovascularisation. TSPs -2 also inhibits the migration and proliferation of endothelial cells in vitro. In the study by Chin- Wen Hsu et al in 2009 <sup>24</sup> reported that proliferation of oral cells significantly decreased when treated with high concentrations of PRP, because TSPs -1 levels will also be high and also may contribute to the antiproliferative effect.

## Conclusion

Bone augmentation in the field of Oral and Maxillofacial Surgery is often performed in various situations such as, after periapical surgeries, impacted third molar surgeries, to improve the recipient site for placement of dental implants and also when creation of a large sized defect is expected after the surgery. This is achieved best with autogenous bone grafts. But this is associated with further operation sites for harvesting bone, longer operation time, potential complications and patients morbidity. To overcome these disadvantages, alternative materials were developed.

The present study showed earlier trabecular bone formation and good amount of blending of margins by the end of 6<sup>th</sup> month. This improvement in bone regeneration signifies and highlights the use of PRP as a valid method in healing of bony defects.

Our study was done with a follow up of 6 months and included only periapical bony defects with small sample, but the effect of PRP can really be tried upon larger defects to assess their potential for osseous regeneration with larger sample and longer follow ups.

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