



**USE OF AUTOLOGOUS PLATELET RICH FIBRIN (PRF) AND ITS COMBINATION WITH HYDROXYAPATITE AS A REGENERATIVE MODALITY IN ENDODONTICALLY INDUCED PERIAPICAL LESION. A CASE SERIES**

**Harsh B Unadkat<sup>1\*</sup>, Gayatri Pendse<sup>2</sup>, Manish Pendse<sup>3</sup> and Lalitagauri Mandke<sup>4</sup>**

<sup>1</sup>Department of Conservative Dentistry And Endodontics, Bharati Vidyapeeth Dental College and hospital, Pune, 411043, India

<sup>2</sup>Department of Conservative Dentistry and Endodontics, Dr.D.Y.Patil school of Dentistry, Nerul, Navi Mumbai, 400706

<sup>3</sup>Department of General Medicine, Dr.D.Y.Patil Medical College and Hospital, Nerul, Navi Mumbai, 400706

<sup>4</sup>Department of Conservative Dentistry and Endodontics, Dr.D.Y.Patil School of Dentistry, Nerul, Navi Mumbai, 400706

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**ABSTRACT**

**Introduction:** Platelet-rich fibrin (PRF), a second generation platelet concentrate, is a cicatricial matrix in which platelets, cytokines and cells are trapped which are released over a certain time and has the potential to accelerate soft and hard tissue healing. It does not require addition of anti-coagulant, thus the chances of cross-infection are less. Also it is inexpensive and requires less chair side preparation time. The following case series explores a novel autogenous method for Regeneration using PRF, PRF and hydroxyapatite in endodontically induced cystic lesions.

**Method:** Necrotic permanent anterior teeth of three systemically healthy patients between ages 20 to 40 years were treated by conventional orthograde root canal therapy followed by periradicular surgery. The osseous defect was filled with PRF in one case and PRF with hydroxyapatite in other two cases. Clinical and radiographic follow up for 6 months was done post-surgically.

**Results:** All the 3 lesions demonstrated radiographic and clinical evidence of favourable hard and soft tissue healing. There was evidence of considerable bone fill and absence of clinical symptoms.

**Conclusion:** Results of this case series show that PRF or PRF with Hydroxyapatite can prove to be an effective regenerative treatment modality for periapical cystic lesions.

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**INTRODUCTION**

Endodontic therapy is aimed at removing the pathologic disease process and regeneration of the surrounding tissues. In endodontics, regeneration relates to formation of new bone, cementum, and periodontal ligament in and around diseased root apex. It is the reproduction or reconstitution of a lost or injured part<sup>1</sup>. Although non-surgical endodontic treatment is always preferred, some periradicular lesions like cysts or lesions with large osseous defects require surgical intervention.

Development of bioactive additives that will regulate inflammation and promote accelerated hard and soft tissue healing has been amongst the great challenges in tissue regeneration. Concentrated platelet rich plasma (cPRP), an autologous fibrin adhesive has the capacity to accelerate

healing and reducing postoperative hematoma. It involved the addition of bovine thrombin or calcium chloride as anticoagulants.

It has been discovered that use of bovine thrombin may be associated with development of antibodies to the factors-V, XI, and thrombin resulting in the risk of life threatening coagulopathies<sup>2</sup>. A new family of platelet concentrate, which is neither a fibrin glue nor a classical platelet concentrate, appeared in France. This new biomaterial is called platelet-rich fibrin (PRF) (Choukran *et al*).

PRF was initially used in oral and maxillofacial surgery, later its applications extended considerably to periodontics and endodontic surgery. This autologous matrix is a rich source of growth factors and its application is thought to be an effective way of inducing tissue repair and regeneration. It has the

ability to stimulate a cascade of healing events, which can result in the excellent osseous defect fill in as early as 6 months on radiographic evaluation. PRF has been successfully used with bone grafts like -TCP for bone regeneration in the treatment of periodontal defects<sup>3</sup>.

## MATERIALS AND METHODS

The protocol for the preparation of platelet rich fibrin was as follows:

A blood sample was taken without anticoagulant in 10-mL tubes, which were immediately centrifuged at 3000 rpm (approximately 400g according to our calculations) for 10 minutes. A fibrin clot was then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top. Platelets are theoretically trapped massively in the fibrin meshes<sup>4</sup>. This paper aims at presenting three case reports in which Platelet rich fibrin (PRF) was used as an autologous matrix for defect fill. In two of the cases hydroxyapatite was also used along with PRF for the purpose of achieving a greater bone fill owing to the defect being larger than what it seemed to be radiographically.

### Case Report 1

A 30 year old female patient came with a chief complaint of pain and pus discharge in relation to tooth 12.

On clinical examination, a sinus tract was observed in relation to 12. On vitality testing tooth 12 was nonvital whereas 11 and 13 were vital. Patient had no history of trauma.

Radiographic evaluation showed the presence of large periapical cystic lesion in relation to tooth 11, 12 and 13. (FIG 1) Tooth 12 was diagnosed as Type II Dens Invaginatus. Root canal treatment was initiated in 11, 12, 13. Access opening of 12 revealed 3 orifices. Since Orthograde three dimensional obturation would be difficult to achieve because of the anatomic variations of dens invaginatus and failure to achieve apical patency from all the three canal orifices, it was decided to treat surgically with curettage & retrograde Mineral Trioxide Aggregate (MTA).

The root canal therapy was completed in 11 and 13 since dry canals existed and tooth 12 was obturated on the day prior to surgery.

An ethical clearance was obtained from the institutional ethical committee.

After patient's consent was taken, complete hemogram was done and all the parameters were found to be within normal limits.

A full thickness mucoperiosteal flap was reflected and complete enucleation of the cystic lesion was done followed by thorough debridement of the surgical site with sterile saline solution.

At this stage, intravenous blood (by venipuncturing of the antecubital vein) was collected in a 10mL sterile tube without anticoagulant and immediately centrifuged at 3,000rpm for 10 minutes. Blood centrifugation allowed the formation of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (platelet-poor plasma) at the top. PRF was easily separated from red corpuscles base (preserving a small RBC layer) using sterile tweezers just after removal of PPP (platelet-poor plasma) and then transferred into a sterile dappen dish. (FIG.4)

Retrograde MTA filling was done in relation to 12 to seal the apical root resorption caused by the disease process. PRF that was prepared earlier was compacted into the bony defect. Wound closure was obtained with a 3-0 black silk suture. Analgesics and antibiotics were prescribed along with 0.2% chlorhexidine mouthwash for 5 days post surgically. Follow up was done for 6 months with uneventful healing. Patient was asymptomatic and clinically good soft tissue healing was seen with complete closure of sinus tract. Radiographically appreciable bone filling was seen at 3 and 6 months.



Fig.1

### Case Report 2

A 23 year old male Patient came to the department of endodontics with a chief complaint of pain in the upper right region since many months. Pain was intermittent, dull and localized. Patient gave a history of trauma (accidental fall) 9 yrs back. Patient had no relevant dental or medical history. On clinical examination and vitality testing 11 and 12 were nonvital and tender on percussion with slight discoloration of 11. Radiographic examination revealed periapical radiolucency extending from 11 to 13. (FIG 2)

Diagnosis made was pulp necrosis with a periapical abscess. Ethical clearance and consent was obtained from the patient. Patient's complete hemogram was done. Due to the time constraint as the patient demanded immediate treatment, periapical surgery was planned.

Access opening followed by instrumentation was done for 11,12. After thorough irrigation, obturation was done one day prior to the surgery. Patient was premedicated with analgesic and antibiotic coverage. A trapezoidal flap was reflected with 2 vertical incisions from 21 to 14. Osteotomy was done with a round bur with a creation of bony window. Since the lesion involved the lateral surface of 13, root canal treatment was performed during the surgery Curettage was done till all the granulation was removed. At this stage patient's blood was drawn and sent for centrifugation. This was followed by root resection and root end preparation and filling of 11 and 12 with MTA. PRF was obtained in 10 minutes and was immediately placed with hydroxyapatite crystals. The flap was approximated and interrupted sutures were given.

Postoperative care instructions were given. Patient was recalled after 7 days for suture removal and every month for a follow up upto 6 months. The healing was uneventful with appreciable radioopacity of the osseous repair at the end of 6 months. Clinically patient was asymptomatic with good soft tissue healing.

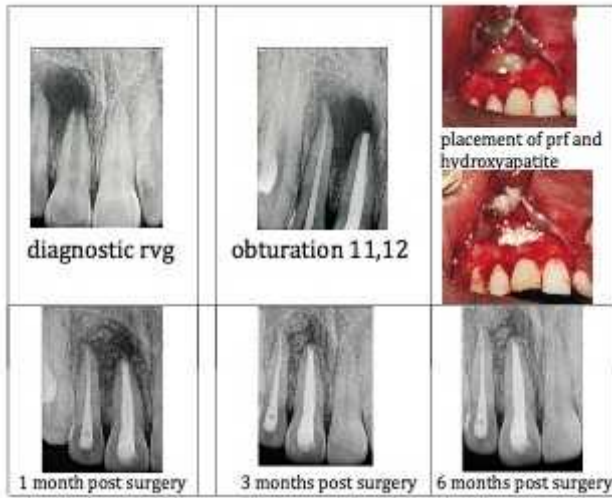


Fig.2

**Case Report 3**

Patient was referred to the department of endodontics with fractured tooth 11 and swelling in relation to the same. Patient gave a history of trauma and had a past dental history. 11 and 12 were tender on percussion. A draining sinus was seen in relation to 11. Radiographic examination revealed a well defined periapical radiolucency with a radiopaque border extending from 11 to 13. (FIG3)

Conventional Root canal treatment of 11,12, 13 was done . Orthograde application of metapex was done for a period of 2 months. As the lesion had not resolved and patient was symptomatic , a periradicular surgery was planned The surgery was performed in a similar way as the previous case with placement of PRF and hydroxyapatite. Follow up was done for 6 months at the end of which an appreciable healing of soft and hard tissues.

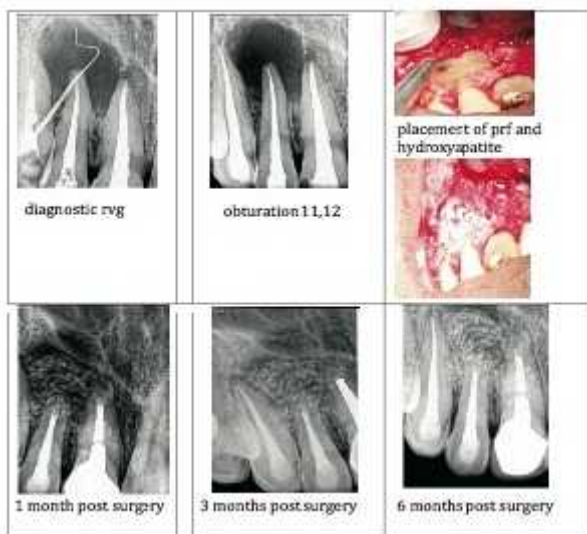


Fig.3



Fig.4

**DISCUSSION**

Unarguably, the best material to replace tooth structure is tooth structure itself. Regenerative endodontics aims at reproducing or reconstituting the lost or diseased tooth structure and its associated tissues. In periradicular lesions with large osseous defects, bone regeneration presents a major challenge even to a skilled clinician, as it is a slow process. Hence a variety of bone replacement materials are being used. The commonly available bone grafts like freeze dried bone graft, bioactive glass, emdogain, PTR polymer, Mineral trioxide aggregate, Tricalcium phosphate and octacalcium phosphate are either highly osteoconductive or osteoinductive.<sup>5,6,7</sup>

It's a widely known fact that Blood and blood products are used for various purposes in the medical field. The functions of platelets like hemostasis and platelet plug formation as well as the use of its growth factors led to its research in regenerative therapy.<sup>8,9</sup>

Platelet rich plasma (Whitman *et al*, 1997) was obtained by sequestering and concentrating platelets by gradient density centrifugation (Marx *et al*, 1998). It contains various growth factors like PDGF, transforming growth factor beta, insulin – like growth factor and epidermal growth factor all of which help in bone regeneration.<sup>9,10</sup>.To overcome the disadvantages of PRP, research led to the use of PRF in the field of dentistry.

Platelet rich fibrin is a second generation platelet concentrate. It's an autologous cicatrical matrix in which platelets, cytokines and cells are trapped within the fibrin network, which are released over a certain time.

The absence of anticoagulant implies that most of the platelets get activated within a few minutes and thus the release of coagulation cascades. Platelets are acellular structures formed in the bone marrow by megakaryocytes with a life span of 8-10 days. They contain granules that release its contents at the time of activation. a-Granules contain many proteins, platelet specific (such as b-thromboglobulin) or nonplatelet specific (fibronectin, thrombospondin, fibrinogen, and other factors of coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins, etc.)(Choukran *et al*). Release of cytokines following degranulation also stimulates cell migration and proliferation within the fibrin matrix thus initiates healing.<sup>11</sup>

Platelet rich fibrin has its own advantages: Firstly, it does not require the addition of any anticoagulant like bovine thrombin, thus eliminating the chances of cross infection, also chair side preparation time is quite easy and fast. (Dohan *et al* 2006). It can be squeezed to form a membrane that can be used as fibrin bandage. (Gabing *et al* 2009, Venice *et al* 2009). Most importantly it is an inexpensive source for osseous fill as compared to bone grafting materials.

PRF accelerates soft and hard tissue healing (Choukran *et al* 2001, Dohan *et al* 2007). Also these results were in accordance with the results obtained by a study done by Simon *et al* (2009). Extraction sockets treated with PRF membrane showed considerable bone fill by 3 weeks.<sup>11</sup>

In vitro studies demonstrate that high concentrations of non activated functional intact platelets contained within a fibrin matrix released a relatively constant concentration of growth factors platelet derived growth factors (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), immunoglobulin growth factor (IGF) over a period of 7 days. (Caroll *et al* 2005)

PRF and -Tricalcium Phosphate allograft were used for periapical healing and this combination accelerated regenerative capacity of bone.<sup>3</sup>

DENS INVAGINATUS is a malformation occurring in permanent, deciduous or supernumerary teeth with a prevalence of 0.25-10 percent. Pulpitis and pulp necrosis are frequently associated with this anomaly. Varied treatment options being: A, preventive treatment. B. endodontic treatment. C. periradicular surgery. D. Intentional replantation. E.Extraction.<sup>12,13,14</sup>

In case 1, tooth 12 was clinically healthy, caries-free, the etiology of the chronic periapical lesion was pulp necrosis caused by the passage of bacteria through the invagination.

Hydroxyapatite works on the principle of osseointegration where in the bone is formed by the ingrowth of capillaries and osteoprogenitor cells from the recipient bed into, around and through a graft. It acts as a scaffold over which new bone is deposited.<sup>15</sup>

In case 2 and 3 since the osseous defect was too large and 2 vials of platelet rich fibrin had already been placed, hydroxyapatite was used to aid in additional bone fill.

Active formation of osseous tissue at the site of cysts, in the periodontal osseous pockets, and at the apexes of interdental septa was observed in 6 to 7 months after treatment when hydroxyapatite and collagen were used for osseous defect fill. (Vorob'ev IuI *et al* in 1995)

Though a long term follow up period with greater sample size is required to conclude PRF and its combination with hydroxyapatite as a Regenerative Technique, nevertheless the results observed in the above 3 cases showed promising results and definitely points out to the use of Platelet Rich fibrin emerging as an effective autogenous modality for regenerative therapy.

## CONCLUSION

Encouraging results were seen in all the three cases. Excellent soft tissue response was seen on clinical examination 1 week post surgical intervention along with appreciable bone fill on radiographic examination at the end of 6 months. PRF is fast

emerging as a new avenue in the era of regenerative endodontics.

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