

**Regeneration in the Horizon - Case Series Using Platelet Rich Plasma and Platelet Rich Fibrin in Traumatized Immature Permanent Teeth**

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**Abstract**

Endodontic treatment is a modality that involves removal of contaminated tissue from teeth with necrotic pulp and replacement with an inert material. This treatment has been known for many years as predictable and reliable. However, immature teeth with a necrotic pulp and apical periodontitis present multiple challenges to a successful outcome. In 2001, Iwaya et al. first reported a case involving the revascularization of an immature tooth with apical periodontitis. Since then, a paradigm shift has occurred regarding the treatment of immature permanent teeth with pulp necrosis or apical periodontitis. In the past decade, several reports have described regenerative endodontic treatment (RET). RET is based on the concept of tissue engineering, which requires the eradication of

pathogens, the preservation of stem cells, and the presence of scaffolds and signalling molecules. In recent years, platelet concentrate, Platelet Rich Plasma and Platelet Rich Fibrin have been successfully applied as scaffolding in tooth revascularization/revitalization. This article presents a series of 3 cases, one which is treated using Platelet Rich Plasma (PRP), and the other two using Platelet Rich Fibrin (PRF) as scaffolds. This case series reports successful application of regenerative endodontic procedure by using PRP and PRF in three traumatized immature necrotic permanent teeth.

**Keywords:** Traumatized teeth, Necrotic Pulp, Immature Teeth, Regenerative Endodontic Treatment, Platelet Rich Plasma, Platelet Rich Fibrin.

## **Introduction**

Dental trauma occurs frequently in children and often can lead to pulpal necrosis. Population-based studies from around the world indicate that the prevalence of dental trauma injuries is about 4%–59%, with the majority of cases occurring in incisors.<sup>1</sup> In treating the immature tooth with pulpal necrosis, the ideal clinical outcomes would be to prevent or heal the occurrence of apical periodontitis, promote continued root development, and restore the functional competence of pulpal tissue, particularly from both immunologic and sensory perspectives.<sup>2</sup> In the last decade, the endodontic profession has witnessed a remarkable shift toward biologically based treatment strategies that aim to promote revitalization and root development in necrotic immature permanent teeth. Regenerative endodontic procedures (REPs) are defined as “biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex.<sup>3</sup> Development of platelet concentrating technique and enhanced efficacy of protocols lead to production of Platelet Rich Plasma (PRP) but due to limitation, a new generation of concentrating platelets that was nor fibrin glue neither concentrating platelet, was introduced in France. This new product named Platelet Rich Fibrin (PRF) was like an autologous cycatrical matrix.<sup>4</sup> Thus, Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) are 2 concentrated sources of platelets that have been used in the field of regenerative endodontics. PRP represents the first generation of autologous plasma and has a 5 times more greater platelet concentration than whole blood.<sup>5,6</sup> Platelet Rich Plasma can deliver an increased number and concentration of growth factors that can stimulate the regeneration process in hard and soft tissues.<sup>7,8</sup> PRF, when used as a scaffolding material in an infected necrotic immature

tooth for pulpal regeneration and tooth revitalization, satisfies many criteria of an ideal physical scaffold. Another advantage of using PRF as a scaffold is that it has a trimolecular or equilateral fibrin branch junction that makes its architecture flexible and can support cytokine enmeshment and cellular migration.<sup>9</sup> The core principles of tissue engineering, namely that tissue regeneration requires an appropriate source of stem/progenitor cells, growth factors, and scaffolds to control the development of the targeted tissue.<sup>10</sup> The gold standard for in vivo tissue healing and regeneration requires the mutual interaction between a scaffold (fibrin matrix), platelets, growth factors, leukocytes, and stem cells.<sup>11</sup>

## **Case Series**

The patients selected were ranging from the age group of 9 years to 15 years with a history of trauma. A clinical diagnosis was made on the basis of a negative response to cold testing with ethyl chloride spray and electric pulp testing. Radiographic diagnosis was made on the basis of incomplete closure of the apex and if it is accompanied by any periapical pathology. Regenerative endodontic procedure was planned only after the consent from the parents was obtained. At the first visit, under local anaesthesia with 2% lidocaine and 1:100,000 adrenaline and isolation with rubber dam, the access opening was performed. Pulp extirpation was done using #20 barbed broach. The root canals were gently irrigated with 20 ml sterile normal saline. Minimal instrumentation was performed using #40 K File. The canals were dried with sterile paper points. Triple antibiotic paste was prepared using doxycycline (Doxy Plus 100 mg), ciprofloxacin (Cipro 200 mg) and metronidazole (Flagyl 500mg) in equal ratio. The enteric coating of the tablets were scraped off and for the capsule, the outer capsular material was removed followed by the tablets being crushed using a mortar and pestle. This powder was mixed with propylene

glycol which was placed in the pulp chamber using a lentulospiral. Dentin bonding agent was applied at the orifice opening and was cured, thus blocking the dentinal tubules so as to prevent the discolouration of teeth due to the triple antibiotic paste, followed by sealing the orifice with at least 4mm thick conventional glass ionomer cement. The patients were recalled after 3 weeks. In cases of persistent signs of infection, the irrigation and medication procedures were repeated for another 4 weeks. On the day of placing the scaffold, irrigation was done with 20ml of 17% EDTA (DPI,17%EDTA) followed by a final rinse with sterile normal saline. In the patient, in which Platelet Rich Plasma was used, 5 mL blood was drawn from the patient's median cubital vein from the right forearm (Figure 1) and collected in 2 anticoagulant coated test tubes for Platelet Rich Plasma preparation, Blood was centrifuged in a standard laboratory in a centrifugation machine (R-8C-REMI) at 900 Revolutions Per Minute for 15 mins- 1st spin and 1250 Revolutions Per Minute for 15 mins- 2nd spin to obtain Platelet Rich Plasma. (Figure 2 and 3). The PRP was injected into the canal space using sterile needle tips to a level of approximately 3 mm less than the radiographic working length determined. A coronal barrier was placed using MTA (Dentsply ProRoot) followed by placement of Glass Ionomer Cement (GC Gold Label High Strength Type-IX) and final coronal restoration with Composite (Brilliant NG Coltene) was done. In the patients in which Platelet Rich Fibrin was used, 5 mL blood was drawn from the patient's median cubital vein from the right forearm and was collected in a sterile test tube without anticoagulant coating and centrifuged immediately at 1600 Revolutions Per Minute for 12 mins. The PRF was extracted from the test tube (Figure 4), and the excess red blood cells were removed with sterile gauze pad. The prepared PRF was then placed incrementally using a sterile tweezer and a

hand plugger, in the canal space to a level of approximately 3 mm less than the radiographic working length determined. The MTA (Dentsply ProRoot) coronal barrier was sealed with Glass Ionomer Cement (GC Gold Label High Strength Type-IX) and final coronal restoration was done with Composite (Brilliant NG Coltene). After follow up of 3 months, in case of Platelet Rich Plasma, minimal apical barrier was noticed with 11 (Figure 5 and Figure 6), whereas in the 1st case of Platelet Rich Fibrin, barrier formation was seen with 21 (Figure 7 and Figure 8) and in the 2nd case of Platelet Rich Fibrin, reduction of periapical infection followed by barrier formation was seen with 22 (Figure 9, 10 and 11). The follow up of these patients were done at 3,6 and 9 months. The patients are under further follow up for the completion of apical development and increase in root dentin thickness, after which the final prosthesis will be delivered in the form of zirconia crown prosthesis.

### **Discussion**

Endodontics has come a long way since 1687 when Charles Allen authored the first book in the English language exclusively on the subject of dentistry describing the techniques of dental transplant.<sup>12</sup> Utilizing the reparative capacity of the dentino-pulpal complex for the treatment of pulpal and periapical pathologies has become the-holy grail of endodontics. This has been made possible because of rapid development in the field of regenerative medicine and tissue engineering.<sup>13</sup> Revascularization is the most studied and successful approach of regenerative endodontics.<sup>14</sup> Badade et al. (2016)<sup>15</sup> evaluated antimicrobial efficacy of PRP and PRF. He found that PRP is more effective against bacteria than PRF. This finding was further confirmed by Kour et al. (2018)<sup>16</sup>. Possible explanation that PRF have subordinate concentration of platelets and leukocytes when matched to the PRP. Furthermore, in PRP, platelets and cytokines

would be completely released once the fibrin meshwork disintegrates. On the other hand, PRF provides a delayed and sustained release of growth factors, as opposed to the single sharp burst of growth factors provided by PRP.<sup>17</sup> Revitalization of necrotic infected immature tooth is possible under conditions of total canal disinfection combined with the additive effect of PRP/PRF. PRF is proposed as an ideal biomaterial for pulp-dentin complex regeneration because it is a potentially valid scaffold material containing leukocyte and growth factors to facilitate tissue healing and regeneration in immature necrotic teeth in children.<sup>18,19</sup> The 3D architecture of the fibrin matrix provides the PRF membrane with great density, elasticity, flexibility and strength that are excellently suited for handling, manipulation and suturing. Another advantage of using PRF as a scaffold is that it has a trimolecular or equilateral fibrin branch junction that makes its architecture flexible and can support cytokine enmeshment and cellular migration.<sup>9</sup> One of the clinical limitations to note is the heterogeneity in the quality of platelets and blood components due to use of different PRF preparation protocols in the various studies reviewed. Irrespective of the protocol used all studies have all reported successful outcomes with regards to soft and bone tissue healing and regeneration.<sup>20</sup> EDTA has weak antimicrobial activity, but is capable of inhibiting biofilm formation.<sup>21,22</sup> Yamuchi et al.<sup>23</sup> and Galler et al.<sup>24</sup> reported that EDTA may encourage dentin-pulp regeneration and enhance the attachment of newly formed tissue to the canal walls by exposing the dentin matrix and cause release of growth factors from the dentin matrix reservoir. Martin et al. recommended use of 17% EDTA in regenerative endodontics as it reverses the deleterious effects of NaOCl.<sup>25</sup> Once a scaffold has been produced within the canal, a bacteria-tight seal is indicated. MTA is currently the material of choice for achieving coronal

sealing in regenerative procedures.<sup>26</sup> Thus the success of this technique is dependent on the fast collection of blood sample and its transfer to the centrifuge. Without use of anticoagulants, the sample should be centrifuged within minutes otherwise the fibrinogen cannot be concentrated in the middle and upper part of tube. It is necessary to concentrate fibrinogen before thrombin convert it into fibrin. Hence, the fast preparation of this protocol is the key tip to achieve a useful PRF clot. The most important property of this technique is not usage of anticoagulants that leads to extensive platelet activation and release of large amounts of cytokines that these soluble molecules trapped into fibrin network.<sup>27</sup>

### Conclusion

It has been reported that after the placement of Platelet Rich Plasma, the maximum release of growth factors is found on the 1st day followed by a continuous decline until the 14th day. Since the ingrowth of cells into the root canal could occur after 14 days, the efficacy of growth factors from Platelet Rich Plasma, for the formation or thickening of dentin is limited. Platelet Rich Fibrin is associated with the slow and sustained release of growth factors for up to 28 days, with peak levels at around the 14th day leading to favourable results in regenerative endodontic procedures. Thus further studies and research is indicated to check the efficacy and comparison of Platelet Rich Plasma and Platelet Rich Fibrin, which will help in our regular clinical practice in treating the traumatized necrotic immature permanent teeth, with one of the limitations of this procedure being long term follow up of the patients.

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### Legends Figure

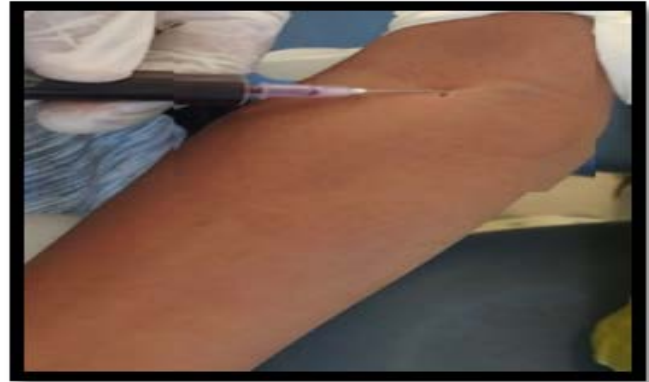


Figure 1: Withdrawal of blood from the patient's median cubital vein from the right forearm



Figure 2: Laboratory Centrifugation Machine

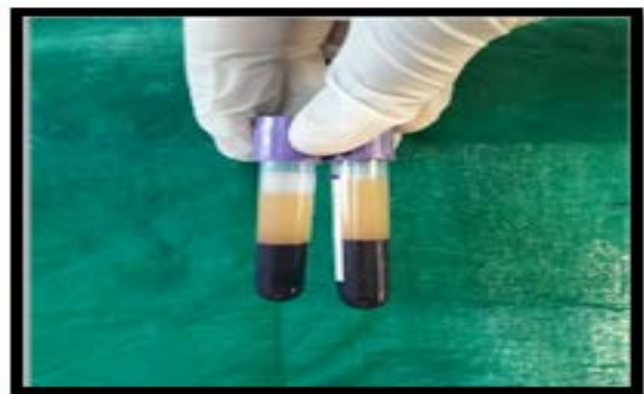


Figure 3: After the blood was centrifuged

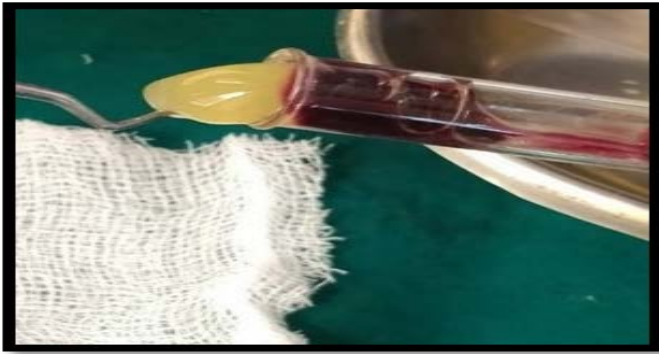


Figure 4: Extraction of Platelet Rich Fibrin

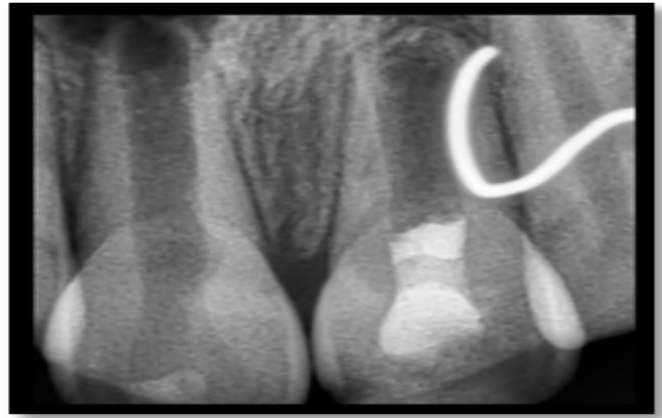


Figure 8: Follow up IOPA with 21 (Treated with PRF)

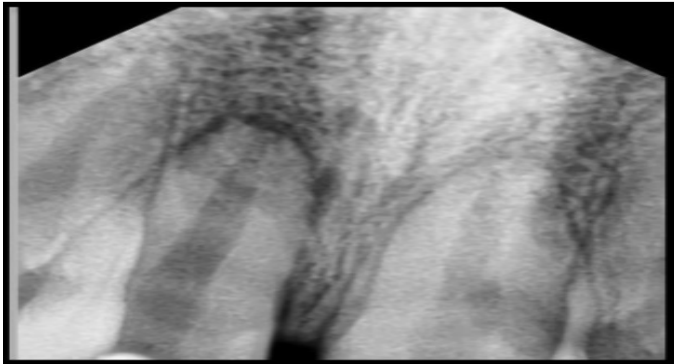


Figure 5: Pre Operative IOPA with 11

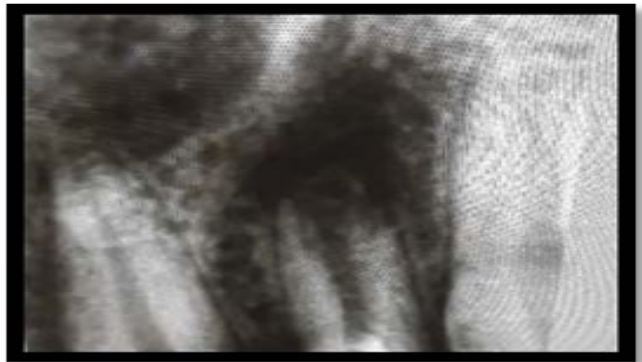


Figure 9: Pre Operative IOPA with 22

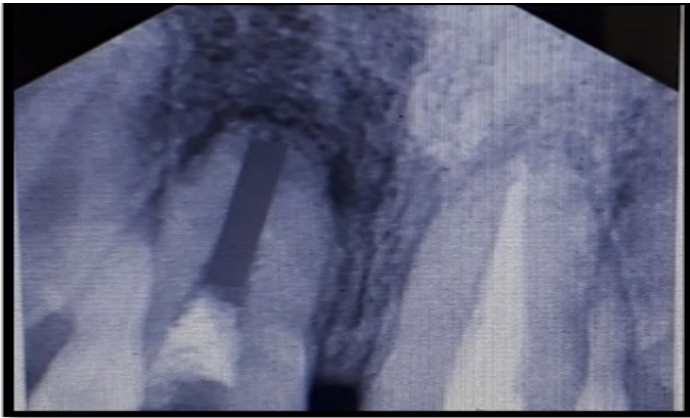


Figure 6: Follow up IOPA with 11 (Treated with PRF)

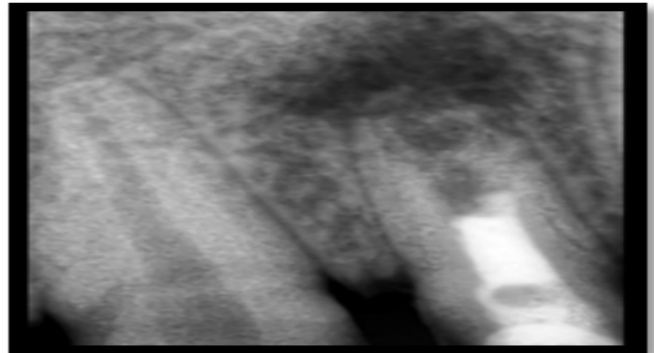


Figure 10: Follow up IOPA with 22 (Treated with PRF)

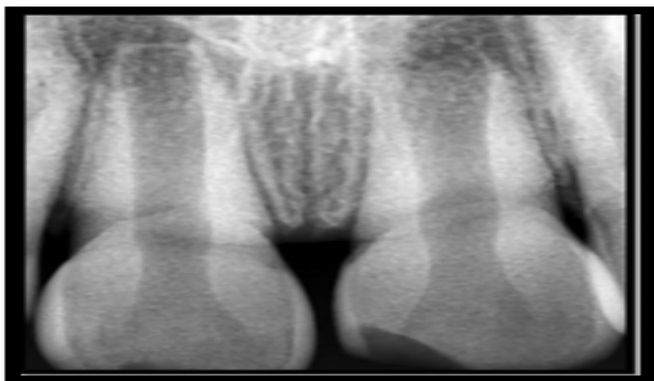


Figure 7: Pre Operative IOPA with 21

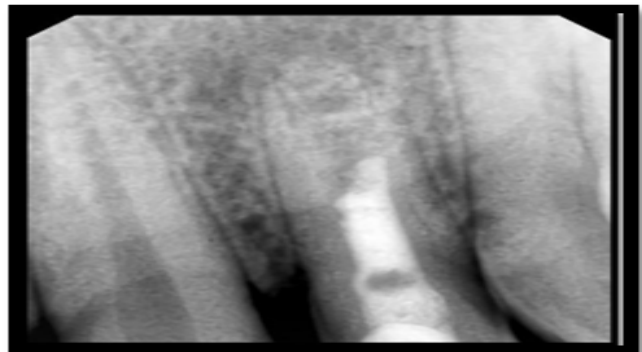


Figure 11: After Further Follow-up IOPA with 22