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# Platelet Rich Fibrin: A New Horizon In Pulp Revasularisation

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**ABSTRACT**

Revascularization is a new approach for treatment of immature necrotic permanent teeth. Till now, apexification procedures were used for these teeth, using calcium dihydroxide or MTA to produce an artificial apical barrier.The pulp revascularization allows the stimulation of the apical development and the root maturation of immature teeth . Platelet rich fibrin (PRF) is a fibrin matrix in which platelet cytokines, growth factors, and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane. Choukroun et al were the pioneers for using PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. Autologous PRF is considered to be a healing biomaterial, and presently, studies have shown its application in various disciplines of dentistry.

**Keywords:** Immature teeth, Platelet rich fibrin, Revascularisation

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## INTRODUCTION

Recently regenerative endodontic procedures are used for the management of immature permanent teeth with necrotic pulp secondary to trauma or caries in children .To treat immature teeth with the conventional techniques is difficult because of thin dentinal walls, open apices, and wide root canal spaces (1) . It is not wise to remove dentine by mechanical cleaning with instruments because it may further break the already thin root canal walls [1] . Conventionally , immature teeth were treated with apexification by using calcium hydroxide as an intracanal medicament to induce a calcific barrier at the apex before filling the root canals [2] .Calcium hydroxide apexification requires a time period between 3 - 21 months with multiple visits leading to inevitable high costs, poor patient compliance and risks of reinfection as it is difficult to create a long-term seals with provisional restorations [3] . Single step apexification using mineral trioxide aggregate (MTA) can be an alternative method to treat immature permanent teeth which was introduced by Shabahang and Torabinazed [4,5] . But some authors [6,7] showed that one-step MTA apexification does not induce further root development. So there is furthermore risk of future root fracture due to the thin dentinal walls and poor crown-root ratio .Thus we need an alternative to treat such cases. Regenerative endodontic procedures are considered to be a better treatment plan than the conventional apexification . Regenerative endodontic procedures are biologically based procedures designed to restore function of a damaged and non functioning pulp by stimulation of existing stem and progenitor cells present in the root canal under conditions that are favourable to their differentiation [8] .

The pivotal components which are responsible for the successful outcome of this procedures are ; stem cells, growth factors and a and a three-dimensional physical scaffold, without which an empty canal space would not support in growth of new tissues from the periapical area [9] . Stem cells which differentiate and support the continued root development, growth factors are responsible for induction of cellular proliferation and differentiation and an appropriate scaffold to promote cellular growth and differentiation . Other than PRF, a blood clot [10] ,collagen [11] and platelet rich plasma (PRP) [12] have been used as scaffold materials for revascularization. Among them Blood clot or PRP mediated revascularisation has shown successful results, but there is still questionable long-term predictability with moderate anticipated benefits observed with these techniques [8,13,14 ] .

## OPERATIVE PROTOCOL

The success of pulp revascularization treatment depends on three elements: root canal disinfection, the presence of a scaffold (blood clot), and hermetic coronary filling. The quality of root canal restoration is questionable when residual bacteria are present in the canal because these bacteria could proliferate and eventually induce a reinfection. [15,16]Therefore, it is essential to have an immune system of quality, major canal disinfection, and a coronary and apical filling allowing no recontamination. So to obtain a successful root canal restoration by using PRF , the steps are as follows

### First Appointment

- Local anesthesia, dental dam isolation and access.
- Copious, gentle irrigation with 20ml NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., needle with closed end and side-vents, or EndoVac™). Lower concentrations of NaOCl are advised [1.5% NaOCl (20mL/canal, 5 min) and then irrigated with saline (20 mL/canal, 5 min), with irrigating needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues.
- Dry canals with paper points.
- Place triple antibiotic paste (mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 0.1 mg/ml) below the CEJ and seal the pulp chamber with a dentin bonding agent (to minimize risk of staining) .
- Seal with 3-4mm of a temporary restorative material such as Cavit™, IRM™, glass-ionomer or another temporary material.

**Second Appointment** (After one week)

- Access response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment time with antimicrobial, or alternative antimicrobial.
- Under rubber dam isolation reopen the access cavity and irrigate the canal by using saline solution to flush out the antibiotic paste.
- Then dry the canal with paper points.

Preparation of PRF (Fig.1-3) - Draw 5ml of blood intravenously from the forearm (antecubital vein) of the patient using an 18 gauge needle and collected in a sterile plastic vaccutube without adding any anticoagulants. Immediately the tube should be centrifuged at 3000 revolutions per minute for 15 minutes. Then the whole blood will separate into three layers 1) a top layer consisting of straw coloured fluid - platelate poor plasma (PPP) 2) middle layer containing platelet rich fibrin clot 3) a lower layer rich in blood cells. A Pasteur's pipette should be used to discard the straw coloured PPP . A sterile tweezer will be inserted into the test tube to remove the PRF clot. Then the PRF gel should be pressed between the sterile dry gauge to squeeze out fluid to form a PRF membrane. After that the PRF membrane should be rolled to the determined working length and placed inside the canal and should be pushed 1mm beyond the working length and coronally upto the level of CEJ by using an endodontic plugger. Then 3 mms of white MTA will be placed directly over the PRF membrane followed by placement of wet cotton pellet and the tooth will be temporarily restore with cavit.

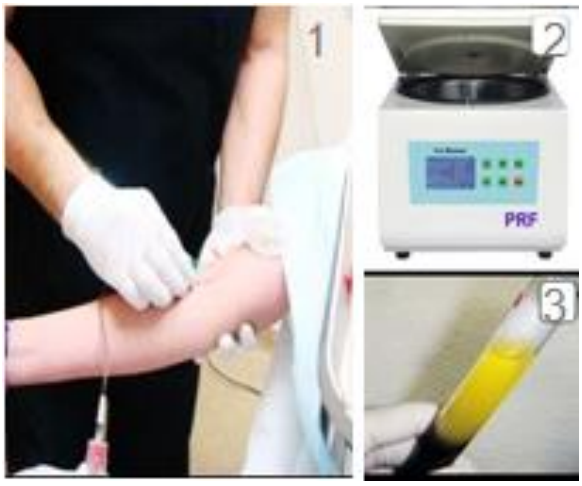


**Figure 1: Collection of blood, blood in vacutainer tube after centrifugation, and centrifuge machine**

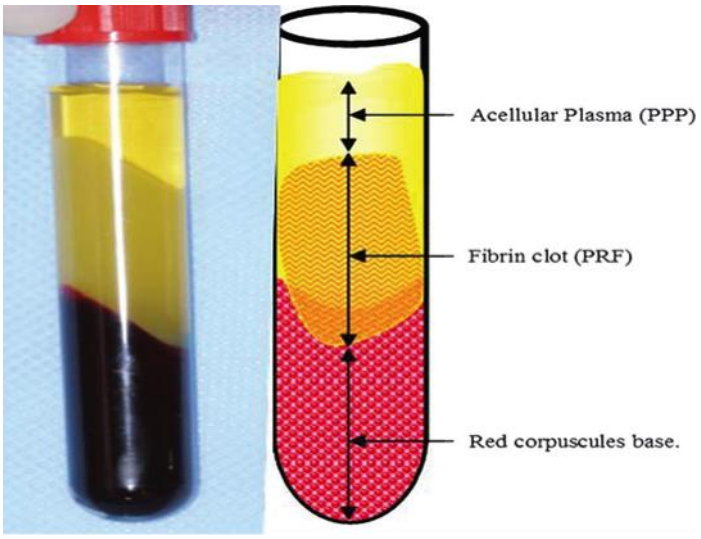


**Figure 2: Blood in the vacutainer tubes after centrifugation at 3,000 rpm for 10 min divided into three fractions; lower fraction of red blood cells, middle fraction containing fibrin clot, and upper acellular plasma fraction.**

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**Figure 3: Isolated platelet rich fibrin**

**Third Appointment** (After one day)

- Patient will be recalled to check the setting of MTA and then the cavity will be restored with glass ionomer cement or composite .

## DISCUSSION

To overcome the problems associated with traditional blood clot or PRP mediated revascularisation, PRF was introduced. Platelet-rich fibrin is prepared naturally without addition of thrombin and has a natural fibrin framework and can protect growth factors from proteolysis. Thus, growth factors can keep their activity for a relatively longer period and stimulate bone regeneration effectively.

**Growth factors released from platelets and their biologic actions p[17]**

| Growth factor | Source cells | Target | Biologic action |
|---|---|---|---|
| Platelet-derived growth factor | Platelets,macrophages, monocytes,endothelial cells, smooth muscle cells, platelets,T-lymphocytes, | Fibroblasts,smooth muscle cells, glial cells, macrophages, neutrophils | Stimulates DNAand protein synthesis in osseous tissues: mitogenic effects on mesenchymal cells;angiogenic effect on endothelial cells |
| Transforming growth factor | Macrophages/monocytes, neutrophils | Fibroblasts,marrow stem cells,endothelial cells,epithelial cells, peosteoblasts | Stimulates angiogenesis; enhanced woven bone formation; stimulate matrix synthesis in most culture systems; stimulates endothelial chemotaxis, stimulates bone formation by inhibitory effect on osteoclasts |
| Platelet- derived angiogenesis factor | Platelets, Endothelial cells, | Endothelial cells | Mitogenic effect on endothelial cells; increased angiogenesis and vessel permeability |
| Insulin - like growth factor1 | Osteoblasts,Macrophages, Monocytes , Chondrocytes | Fibroblasts, Osteoblasts, Chondroblasts | stimulates proliferation of osteoblasts and matrix synthesis; increase expression of bone matrix formation such as osteoclastin in combination with platelet derived growth factor it enhnces the rate and quality of wound healing |
| Platelet factor 4 | Platelets | Fibroblasts,neutrophils | Chemoattraction for neutrophils and fibroblasts |

Various studies [18-20] have demonstrated that the PRF has a very significant, slow-sustained release of many key growth factors, like platelet-derived growth factor and transforming growth factor beta, for at least one week and up to 28 days; this means that PRF could release growth factors with its own biological

scaffold for the wound healing process. The conversion of fibrinogen into fibrin takes place slowly with small quantities of physiologically available thrombin present in the blood sample itself. Thus, a physiologic architecture that is very favorable to the healing process is obtained due to this slow polymerization process. Leukocytes in PRF act as an anti-inflammatory agent, play a key role in immune regulation, and provide vascular-endothelial growth factor to promote angiogenesis [21].

Regarding the root canal temporary medication, triple antibiotic paste covers at best action spectra of root canal bacteria and show minimum stem cells cytotoxicity when used in adequate concentration (0.39 $\mu$g/mL). Among these three antibiotics, minocycline is the one which cause tooth discolouration. Minocycline is a semisynthetic derivative of tetracycline and is effective against gram-positive and gram-negative bacteria . It binds to calcium ions via chelation to form an insoluble complex. Hence, the minocycline incorporated into the tooth matrix causes the discoloration [22] . Therefore, minocycline cannot stain the tooth matrix unless it comes in contact with the coronal dentin. Reducing the application time of the pastes might also prevent discoloration associated with use of minocycline. Sato et al [23] suggested that minocycline should be used only for limited periods and attempted to find substitutes for minocycline in the triple antibiotic paste as a result of the risk of tooth discoloration. They reported that cefaclor and fosfomycin are possible alternatives for minocycline in terms of their antibiotic effectiveness.

The disadvantages associated with PRF are the invasive procedure of drawing of blood and difficulty in handling and placing the PRF membrane inside the root canal, as it is jelly like in consistency and sticks to the instrument.

## CONCLUSION

Platelet-rich fibrin is a potential scaffold in pulp revascularization procedures, as it is rich in growth factors, enhances cellular proliferation and differentiation, augments angiogenesis, acts as a matrix for tissue ingrowth, regulates inflammation reactions and has anti-infective properties. Additionally, it acts as an excellent matrix to support MTA placement. However, long-term clinical trials and histological studies are required to analyse the benefits of using PRF in revascularization procedure.

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