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Comparative evaluation of the efficacy of injectable-PRF and blood clot in maturogenesis of non- vital immature permanent teeth- A clinical study

Running title: Efficacy of I-PRF, PRF on non-vital immature permanent teeth

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Abstract

Introduction: The regenerative endodontic procedure (REP) is considered as a viable treatment option for immature teeth with necrotic pulp caused due to trauma.

Aim: A clinical trial was designed to evaluate the efficacy of blood clot and injectable PRF on root maturogenesis of immature permanent teeth.

Materials and Methods: Ten children of 8-13 yrs. age group were included in the study. After access opening, irrigation with 1% sodium hypochlorite and Tri-antibiotic paste was placed in the teeth. After 3 weeks, the cases were divided into two groups with five patients in each. Group I in which blood clot was induced and Group II injectable PRF was placed in the root canal as a scaffold.

Results: Injectable PRF induced greater root lengthening, shortened the healing of soft and hard tissues than blood clot in root maturogenesis.

Conclusion: Injectable PRF was more effective in root maturogenesis of non-vital immature permanent teeth compared to the blood clot.

Key words: Blood Clot, immature tooth, Injectable PRF, Maturogenesis, Traumatic non-vital,

Introduction:

The management of permanent teeth with periapical pathology, necrotic pulp, and arrested root development creates a great challenge for the dental practitioner. The results of halted root development include weak root dentin, open apices and stunted root growth such teeth are prone to root fracture. Such abnormalities make them unsuitable for debridement and filling with traditional techniques and obturation materials.[1]

Conventionally apexification done usually with calcium hydroxide to form an apical hard tissue barricade in multiple visits. [2] With the advent of mineral trioxide aggregate (MTA), which showed a high success rate in apexification.[3] MTA has also reduced the number of required clinical sessions. A disadvantage common to both calcium hydroxide and MTA, is that they do not allow for continued root development, resulting in thin dentin walls and hence a feeble root structure.[4,5]

To overcome the drawbacks of calcium hydroxide and MTA, various authors have recently reported on the concept of Regenerative Endodontics with newer methods such as using PRF, injectable -PRF (i-PRF), adult stem cells, blood clot, signalling molecules, and a three-dimensional (3D) physical scaffold that can sustain cell growth and differentiation. Unlike apexification, regeneration allows for rapid continuation of root development, increased wall thickness, and natural healing of periapical tissues.[6,7]

Since past two decades, there is an increased understanding of the physiological aspects of platelets in wound healing after tissue injury. This has resulted into an idea of using platelets as a potential scaffold for Regenerative Endodontic Therapy (RET).[7]

The second generation platelet concentrate known as Platelet Rich Fibrin (PRF) which is totally autologous in nature contains platelets, growth factors and cytokines that enhance the healing potential of both soft and hard tissues.[8] The potential applications of PRF as a biologic scaffold to promote the regeneration of lost or injured pulp tissues were mentioned in the research study. [9-10] The PRF technique is very easy to prepare, inexpensive and does not require biochemical modification, and allows the quick production of natural fibrin membranes, enriched with platelets and leukocytes. [11]

Previous studies used blood clot as scaffold with the resultant increase in concentration of growth micromolecules. Blood clot has lower platelet and growth factors compared to PRF. A natural human blood clot contains 95% red blood cells, 5% platelets, <1% white blood cells (WBCs), and various quantities of fibrin strands. Whereas PRP clot, consists of 95% platelets, 4% RBCs, and 1% WBCs. Platelet count in PRP is more than 2 million/μL, hence platelet concentration increases by 160% to 740%, however PRF yields a 210-fold larger concentration of platelets and fibrin in comparison to the initial input whole blood volume. [12]

The PRF can be used as a membrane or a liquid or injectable form (i-PRF) and applied either in standalone therapies (plug, filler or barrier); additive therapies (added or mixed to bone substitutes) or used in combination therapies with other biomaterials (protective barrier in GBR procedures).[13] The i-PRF has demonstrated the ability to release higher concentrations of various growth factors up to 10 days as compared to PRP. [14]

There is lack studies comparing the effect of i-PRF and blood clot in maturogenesis of immature tooth. Hence the present study was carried out to evaluate the efficacy of i-PRF with blood clot in maturogenesis of immature permanent teeth.

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Materials and method

The study was conducted in the Department of Preventive and Paediatric Dentistry from 2018 -2020, after obtaining approval from Institutional Ethics Committee, Pravara Institute of Medical Sciences (IEC-PIMS), Loni, Ahmednagar.

The inclusion criteria were; the paediatric patients between 8-13years of age who were healthy, free of any systemic diseases, patients with non-vital immature permanent single rooted teeth, secondary to trauma and/or caries, preoperative radiograph showing incomplete root formation, with wide apical foramen (apex>/= 1.1mm) and thin dentinal walls.

The exclusion criteria were; patients with medically compromised conditions, patients who are allergic to any drugs, patients with presence of periodontal pockets, pathological mobility, ankyloses, root fracture and presence of periapical radiolucency more than 10mm. The study observations and the clinical outcomes being followed up for 18 months. The study was performed by single trained evaluator.

Clinical Procedure

After considering the inclusion and exclusion criteria, 10 patients were included and divided into 2 groups with 5 patients in each (Group I: blood clot, Group II: i-PRF). Demographic profile, health history and involved teeth conditions were recorded in the Patients Record Sheet. Clinical and radiographic findings were recorded at baseline and at 18 months after treatment.

Pulp necrosis was diagnosed by the clinical examination, which included thermal (cold) and electric pulp testing (EPT). Clinical signs and symptom ms such as pain, swelling and sensitivity to percussion and palpation were noted. Periapical radiographs were taken using CBCT. The baseline findings e.g.- width of apical foramen, dentin thickness, root length, periapical lesion dimensions were recorded in the Patient's Record Sheet.

Preoperative radiographs in all cases showed incomplete root formation, wide apical foramen and thin dentinal walls as per the inclusion criteria. Patients were diagnosed with pulp necrosis and chronic apical periodontitis.

Access opening was done in involved tooth. The canal was instrumented thoroughly with 20ml of 1% sodium hypochlorite solution and dried with sterile paper points. The Tri-antibiotic paste was prepared and placed within the canals below CEJ to minimize crown

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staining. The access cavity was sealed temporarily with GIC (Ketac-Molar 3M ESPE). The patients were scheduled for the second visit after 3 weeks.

After 3 weeks, if tooth found asymptomatic, then further procedure was continued. The antibiotic paste was removed and irrigated with 17% EDTA solution for 5 min. No instrumentation was done and canal space was dried with sterile paper points. After this procedure, the patients were split into two groups; Group I (Blood clot) i.e. Control and Group II (patients being treated with i-PRF).

For Group I, using no # 20 k-file apical bleeding was induced and allowed for blood clot to form till cementoenamel junction. After this, white MTA (Pro root Dentsply) was placed to a thickness of 3mm followed by GIC (GC, Japan) and Composite (Filtek^{TM}) restoration (**Fig1,2).**

For Group II, the I-PRF was prepared using Choukroun's method, where six ml of venous blood was obtained from the antecubital vein of the patient in the Z tube and was centrifuged (Remi 8 M Plus) at 60 G at 700 RPM for 3 min. Freshly prepared i-PRF (0.5ml) was placed in the coronal third of the canal **(Fig 3)**. MTA (ProRoot, Dentsply, Tulsa,USA) was placed in coronal third of the root, restored with GIC, Composite (Z250, 3M ESPE) (**Fig 4)**

All the patients were recalled after 18 months' interval for the clinical evaluation of signs and symptoms, and radiographic assessment of root maturogenesis. Post operatively the degree of success of REP was measured by the guidelines given by American Association of Endodontists (AAE) 2016, that has 3 Goals: Primary goal: The elimination of symptoms and the evidence of bone healing (clinical success), Secondary goal: increased root wall thickness and/or increased root length (Radiographic success) and Tertiary goal: positive response to vitality testing. Clinical success was concluded with absence of pain, soft tissue swelling, or sinus tract**.** Radiographically increased root length, dentin thickness and apex closure was considered as successful **(Fig 2,5).**

Fig-1: Gr I: Pre- operative CBCT

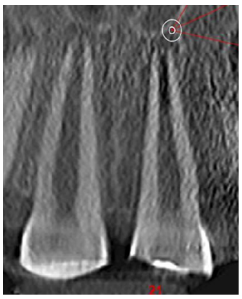

Fig 2: Gr I: Post-operative CBCT showing apical closure and increased dentin thickness

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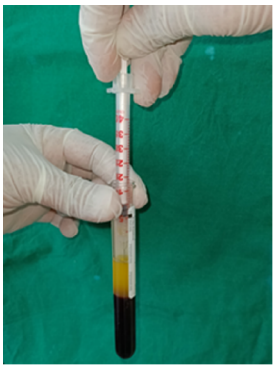


Fig 3: Picture showing collection of injectable -PRF (supernatant coat) using Z- tubes

Fig 4: Gr II: Pre- operative CBCT with wide apical foramen

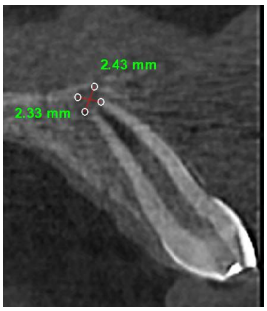
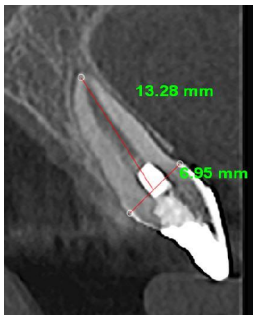


Fig 5: Gr II: Post- operative CBCT showing root lengthening



Statistical analysis: Obtained data was analysed using Statistical analysis software namely SYSTAT version-12 (made by Crane's software) using unpaired 't" test and Chi square test. P value was set at <0.05.

Results

Table No.1: Comparison of Clinical signs and symptoms from Baseline to Postoperative in Group I: Induced bleeding technique (Control group (n=5) of non- vital immature permanent teeth

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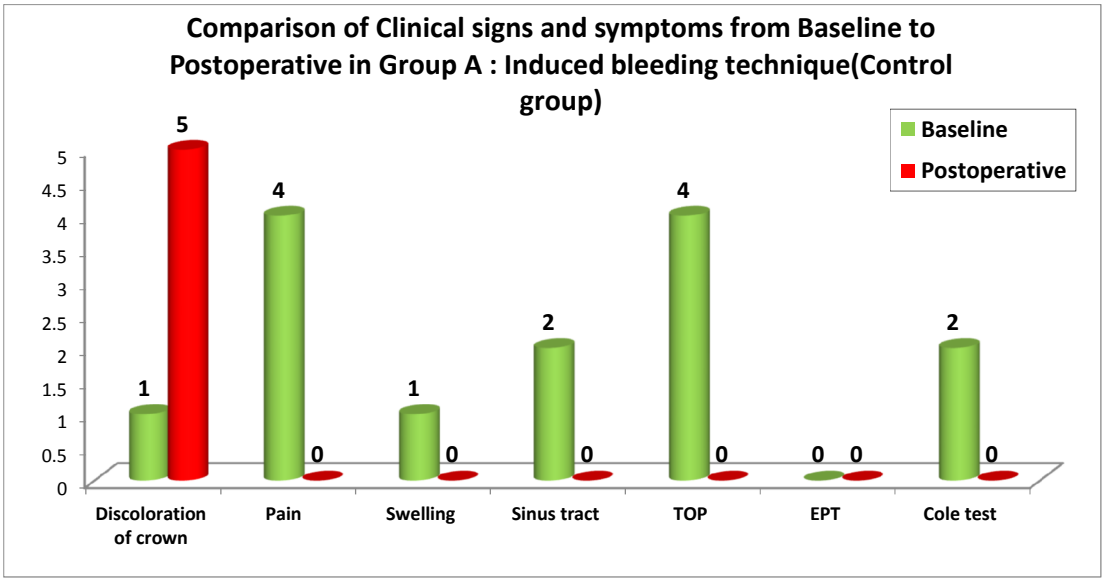
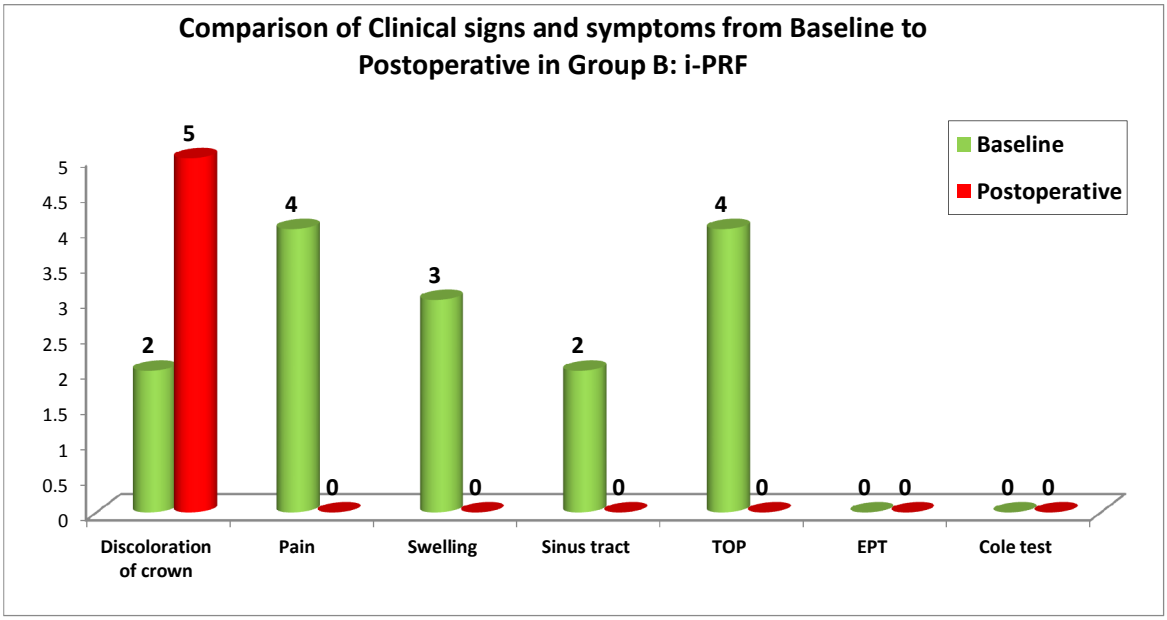


Value of $\square^2$ = 29.874, p=0.001, significant

Table 1 indicates Comparison of Clinical signs and symptoms from Baseline to Postoperative in Group I with Induced bleeding technique. There was decrease in post-operative clinical conditions. By applying Chi-square test there is a significant association between clinical signs and symptoms in Group I.

Table No.2: Comparison of Clinical signs and symptoms from Baseline to Postoperative Group II: i-PRF (n=5) of non- vital immature permanent teeth



Value of $\square^2$ = 29.856, p=0.001 significant, test used: Chi square test

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Table 2 indicates, comparison of Clinical signs and symptoms from Baseline to Postoperative in Group II: i-PRF. There was significant decrease in clinical symptoms post operatively compared to baseline.

Table No.3: Comparison of Radiographs from Baseline to Postoperative among Group I

	Group I: Induced bleeding technique (Control group (n=5)		**Student's Paired 't' test value**	**'p' value and significance**
	Baseline	**Postoperative**		
	Mean ± SD	**Mean ± SD**		
Periapical radiolucency (mm)	2.81±1.14	0±0	6.63	p=0.0001, significant
Width of apical foramen (mm)	1.33±0.31	0±0	5.87	p=0.0001, significant
Root length (mm)	12.68±1.36	13.57±0.98	3.97	p=0.0001, significant
Dentin thickness (mm)	1.26±0.23	2.26±0.72	4.66	p=0.0001, significant

Test used: paired 't' test

Tables 3 indicates, Radiographic evaluation from Baseline to Postoperative among Group I. There was decrease in periapical radiolucency and apical foramen width post operatively (0), increase in mean root length (13.57 mm) and dentine thickness (2.26mm).

Table No.4: Comparison of Radiographs from Baseline to Postoperative among Group II

	Group II: i PRF (n=5)		**Student's Paired 't' test value**	**'p' value and significance**
	Baseline	**Postoperative**		
	Mean ± SD	**Mean ± SD**		
Periapical radiolucency (mm)	2.25±1.27	0.00±0.00	4.01	p=0.0001, significant
Width of apical foramen (mm)	1.34±0.28	0.00±0.00	5.69	p=0.0001, significant
Root length (mm)	11.67±1.04	13.66±1.25	5.53	p=0.0001, significant
Dentin thickness (mm)	1.39±0.23	2.11±0.22	3.99	p=0.0001, significant

Test used: Paired 't' test, highly significant- P=0.001

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Tables 4 indicates, radiographs evaluation from Baseline to Postoperative among Group II. There was improvement in root length (13.66) and dentine thickness (2.11) but decrease in periapical radiolucency (0) and apical foramen width (0) post operatively.

Table No.5: Comparison of Radiographs from Baseline to Postoperative among Group I and Group II

	Group I: Induced bleeding technique (Control group (n=5)		**Group II: i- PRF (n=5)**		**Student's Unpaired 't' test and significance (Postoperative to Postoperative)**
	Baseline	**Postoperative**	**Baseline**	**Postoperative**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
Periapical radiolucency (mm)	2.81±1.14	0±0	2.25±1.27	0.00±0.00	t = 0, p=0.00, not significant
Width of apical foramen (mm)	1.33±0.31	0±0	1.34±0.28	0.00±0.00	t = 0, p=0.00, not significant
Root length (mm)	12.68±1.36	13.57±0.98	11.67±1.04	13.66±1.25	t = 1.33, p=0.1247, not significant
Dentin thickness (mm)	1.26±0.23	2.26±0.72	1.39±0.23	2.11±0.22	t = 1.41, p=0.1493, not significant

Table 5 indicates, comparison of Radiographs from Baseline to Postoperative among Group I and Group II. There was decrease in post-operative radiolucency and apical foramen width in both the groups, which was not statistically significant. In our study dentine thickness was significantly more in group I compared to Group II, whereas root length increase was significantly greater in Group II compared to Group I at 18 months of interval. In both the Group I and Group II, periapical radiolucency has been completely healed and apical closure was seen.

Discussion

Immature non vital teeth management is posing challenges in achieving root lengthening or apical closure in endodontics. Recently regenerative endodontics with PRF; a second generation concentrate is gaining importance. The advantage of regenerative endodontics compared to apexification is that in regenerative endodontics root lengthening and apical closure is possible however with apexification it is not achieved. [15]

PRF scaffold can be used to induce maturogenesis of root. An ultimate scaffold should permit cell localisation, and cell binding, supply of growth factors, and it should be biodegradable in nature. Even though blood clot is a good scaffold, but it is a poor source of growth factors. The concentration of platelet in PRF is five times as higher than that of normal platelet count. It is a concentrated suspension of various growth factors like; transforming growth factors, platelet derived growth factor, insulin like growth factor, vascular endothelial growth factor, epidermal growth factor. PRF is easy to prepare, requires no biochemical handling of the blood and fastens soft and hard tissue healing.[16]

Shivashankar et al evaluated the PRF, PRP and Blood clot in the revascularization of non-vital immature tooth and after 12 months' follow-up, and they observed absence of any pain, swelling and concluded that PRP was better than PRF and induced bleeding technique. It was due to liquid constancy of PRP that enabled it to reach the periapical area without any hindrance. [16] This result was in contrast to our findings, wherein i-PRF showed better efficacy in root maturogenesis than blood clot.

Uppala compared the regenerative efficacy of PRF, blood clot and collagen and found that, PRF and collagen were superior than blood clot in regeneration of tooth similar to our findings. [15] Ulusoy et al assessed the Platelet-rich Plasma, Blood Clot, Platelet-rich Fibrin, and Platelet Pellet as in Regenerative Endodontic and concluded that PRF, PRP, and PP can show comparable clinical and radiographic outcomes but blood clot and PRP had better outcome compared to PRF and platelet pellet.[17] Panda et al in a meta-analysis on Autologous Platelet Concentrates for immature necrotic teeth concluded that, autologous platelet concentrates had better apical closure and improved response to vitality tests.[18]

In both the groups, we did not regain response to the vitality tests. The reason is attributed to the MTA in the coronal third of the root. In our study the follow up was up to 18 months. To get the further results of the vitality regain, the patients are required to be followed up to 5 yrs for the assessment of the vitality.

Miron et al evaluated the effectiveness of i-PRF and observed that i-PRF had the ability to release higher concentrations of various growth factors.[19] Nagaveni et al in a case report observed increased root lengthening and dentin thickness with PRF compared to blood clot. [20] Alrashidi et al from a meta-analysis conclude that blood clot and PRF had comparable apical closure, healing of apical radiolucency, and an increase in root length of nonvital immature permanent teeth. [21] Kalaskar evaluated the maturogenesis of non‑vital immature permanent teeth by inducing blood clot with 3 mix antibacterial paste and found root maturation after 5

month recall check-up. [22] Amit et al similar to our study induced bleeding in canal followed by MTA placement and observed successful outcome with root development after 18 months. [23]

In our study dentine thickness was significantly more in blood clot (group I) compared to i-PRF (Group II), whereas root length increase was significantly greater in Group II compared to Group I at 18 months of interval. The limitation of the study is smaller sample size. Further long term studies are needed on the larger sample size to check the efficacy of blood clot and i-PRF in regenerative endodontics.

Conclusion

Injectable PRF has shown better efficacy in achieving root maturogenesis in non vital immature permanent teeth, compared to the blood clot.

Conflict of interest: Nil

Source of Funding: self

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