ORIGINAL ARTICLE

Pulp Revascularization/Revitalization of Bilateral Upper Necrotic Immature Permanent Central Incisors with Blood Clot vs Platelet-rich Fibrin Scaffolds—A Split-mouth Double-blind Randomized Controlled Trial

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Abstract

Objectives: Clinical and radiographic evaluation of the regeneration of bilateral necrotic upper permanent central incisors with open apex using blood clot (BC) and platelet-rich fibrin (PRF) scaffolds.

Trial design: Split-mouth double-blind parallel arm randomized controlled clinical trial.

Materials and methods: Randomization and blinding: The study started with 15 patients with bilateral necrotic upper permanent central incisors with open apex. Computer-generated tables were used to allocate treatments. The two maxillary central incisors were randomly assigned to either the control (BC scaffold) or the examined (PRF scaffold) groups. Participants: Thirteen patients aged 8–14 years fulfilled the study requirements. Follow-up was performed for 3, 6, 9, and 12 months. Standardized radiographs were collected each 3 months, and difference in measurements was calculated using Image J software. Primary outcomes measured were sinus/fistula formation, pain complaint, mobility grade, and swelling presence/absence. Radiographic: Root length elongation and increase in root thickness. Secondary outcomes were sensibility test and crown color change. Radiographic: Change in bone density and apical diameter. Radiographs that were standardized used during the follow-up time, and occurred changes were calculated using Image J software.

Results: One patient was lost during follow-up; therefore, 24 treated teeth were analyzed, they showed 100% success rate. Platelet-rich fibrin teeth displayed a statistically significant growth in radiographic root length and width, increased periapical bone density, and a reduction in apical diameter when compared with BC. At the end of the follow-up period, all treated teeth were negative to sensibility test. Blood clot displayed greater crown discoloration in comparison to PRF group.

Conclusion: For teeth with open apex and necrotic pulp, revascularization using PRF is an appropriate substitute to BC.

Keywords: Bilateral necrotic upper permanent central incisors with open apex, Blood clot, Platelet-rich fibrin, Revascularization, Revitalization.

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Introduction

Necrotic permanent teeth with open apex with/without periapical pathology are usually treated by apexification with mineral trioxide aggregate (MTA) or calcium hydroxide, and might be disposed to mutilation as a result of seized root maturation due to thin dentinal walls.[1,2]

Since Nygaard-Ostby and Hjortdal[3] and Horsted and Nygaard-Ostby[4] published an article about possibility of tissue formation if bleeding could be initiated inside the root canal system, the treatment of immature permanent teeth has witnessed tremendous change in its concept. From that time, there has been many case reports and case series signifying that regenerative endodontic techniques (RET) can result in clinical and radiographic successful outcomes.[5–7]

Regenerative endodontic techniques main advantages are promoted root maturation in terms of thickness and length.[8] Fruitful RET desire the existence of stem cells, signaling molecules (growth factors), and scaffolds.[9]

Regenerative endodontic techniques protocol utilizing blood clot (BC) as a scaffold, include bleeding initiation from periapical area to form BC inside the root canal, acting as a scaffold for stem cells, fibroblasts, and macrophages migration. Enmeshed cells in the BC scaffold could further discharge signaling molecules fundamental for wound healing.[10] Published data have proved the accuracy of a BC scaffold in regenerative endodontics.[11] Nevertheless, BC creation in certain cases are problematic. Hertwig epithelial root sheath might be damaged in case multiple attempts are exerted to initiate bleeding, which is a decisive component for root maturation. As a result, harm to this vital structure may result in no more root maturation or development of defective root apex.[12] In addition, the encouraged pulp bleeding which may not be the model process or function as a scaffold to attract the adult (pluripotent) stem cells causing unreliable pulp–dentin tissue

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regeneration.[13] Furthermore, a number of authors have reported cases in which it was not possible to produce bleeding in a canal.[14,15] The previously stated concerns have urged researchers to look for better scaffolds that can be constructed regardless of whether or not bleeding can be evoked.

Other scaffold alternatives have been proposed and examined. Being obtained from the same individual and prepared easily, platelet concentrates hold immense combination of signaling molecules. Platelet-rich fibrin (PRF) has many advantages over BC. First, PRF is a coordinated fibrin enmesh leukocytes and platelets, discharging assorted signaling molecules over a long period of time. Second, within PRF cytokines and immune cells are present which could assist during infection.[16] Therefore, better results could be expected when using PRF as a scaffold over BC.

In addition to the above-mentioned points, deficiency of well-designed split mouth randomized controlled clinical trials prevents the widespread application of this promising treatment protocol and also an accurate evidence-based ranking to be established between BC and PRF scaffolds. Therefore, the aim of the study is to, clinical and radiographically assess the regeneration of PRF and BC scaffolds in the regeneration of bilateral necrotic upper permanent central incisors with immature apex.

Materials and Methods

Trial Design

The study is a randomized clinical trial (RCT). Furthermore, it is a double-blind study where participants were blinded to the treatment received. In addition, the outcome analyzer and the person performed the statistical analysis were blinded.

Sample Size Determination

Thirty upper anterior permanent incisors with immature apex in 15 subjects were encompassed. Calculated with method described by Pozos-Guillén et al. and Pandis et al.[17–19]

Study Setting

Children were tabbed from outpatient clinic of Pediatric Dentistry, Faculty of Dentistry, Suez Canal University from January 1, 2015 to May 25, 2017 till predetermined selection criteria were obtained.

Ethical Approval

The (study) research was conducted after it was accepted by the postgraduates studies Committee of the Faculty of Dentistry, Suez Canal University in their session held on January 2016.

Eligibility Criteria[20,21]

Inclusion Criteria

(1) Both gender (male and female). (2) Subjects were free from any chronic systemic disease. (3) Restorable teeth. (4) Bilateral upper permanent incisors with open apex ≥1.0 mm. (5) Necrotic pulp, with or without periapical lesions. (6) Age: 8–14 years.

Exclusion Criteria

(1) Allergic response to any medication or materials used in the study. (2) Teeth never been treated pulpally before. (3) Tooth in question has class III mobility (Miller's classification). (4) Ankylosis identified clinically or radiographically or inflammatory (infection-related) root resorption (external or internal). (5) Radiographic identification of root fracture or abnormality. (6) Uncooperative patients. (7) Consent is not provided.

Clinical Diagnostic Procedure

Diagnostic form was used to document patient data.

Sequence Generation

Fifteen patients initiated the study. Patients allotted numbers from 1 to 15 using computer software (www.random.org). Allocation concealment: the bilateral maxillary central incisors in each group was randomly assigned by a coin toss. Blinding was not possible for the clinician. Informed consent was signed from each participant parents.

Radiographic Diagnostic Procedure and Standardization

Standardized radiographs were obtained using method described by Hamanaka et al.[22] Intervention procedure:[21] first appointment: local anesthesia was conducted using 2% lidocaine (Octocaine 100, Novocol pharmaceutical, Canada), teeth were isolated with a rubber dam (Medium, 6 × 6 inch, CROSSTEX, USA), teeth cleaning and disinfection with, 10% Povidone-Iodine BETADINE antiseptic solution (The Nile Co. for Pharmaceuticals and Chemical Industries—A.R.E), pulp chamber was accessed by round diamond bur (No. 016 long shank, Öko DENT, Germany), and safe tip fissure carbide bur (Endo-z bur, Dentsply, Maillefer, USA). The working length of the canals was determined radiographically 1 mm shorter than the apical foramen and recorded for reference determined using a size 40–90 sterile K-files according to the canal width. Disinfection of the canal was chemical using sodium hypochlorite 2% NaOCl followed by EDTA 17% using side-vented needle. Suitable size paper points were utilized to achieve root canal dryness. Triple antibiotic paste (TAP): ciprofloxacin tablets 250 mg (SPIMACO El-Dwaia Company, Saudi Arabia), metronidazole tablets 250 mg (Sanofi Aventis, Cairo, Egypt), and minocycline capsules 100 mg (Pfizer, India), tablets were grounded individually by pestle in separate mortars producing fine powder, while the minocycline capsule was evacuated from its powder content into separate mortars with a ratio of 1:1:1 by weight with final concentration of 0.1 mg/mL. Then, it was delivered into root canal via a specially designed syringe utilizing tip of pediatric cannula. All the remaining mix was discarded at the end of the session, as it must be freshly prepared for standardization. Cavity was closed using glass ionomer for 3 weeks. Second Appointment: total resolve of signs and symptoms was evaluated. Local anesthesia administration without vasoconstrictor (3% mepecaine, Alexandria pharmaceuticals, Egypt), rubber dam isolation, and disinfection of operating field using betadine, the glass ionomer was removed. Intracanal antibiotic paste was eliminated using physiological saline (sodium chloride 0.9%, FIPCO Egypt) followed by EDTA 17%. After root canal, dryness was achieved, scaffolds were assigned into BC scaffold intervention (control group) and PRF examined group.

BC Scaffold Intervention (Control Group)

Bleeding was induced using a file past periapical area; blood fill the root canal slightly below the cementoenamel junction (CEJ) (Fig. 1). Bleeding was initiated in all cases first for three reasons: (a) to ensure that the bleeding was induced, if no bleeding could be initiated, bleeding was tried in the contralateral tooth. (b) In case bleeding could not be initiated in both teeth, blood was withdrawn from patient and was injected inside the canal. Fortunately, we could initiate bleeding in all cases and no need to withdraw blood from patient. (c) The time taken about 10–15 minutes for clotting to take place was consumed in the preparation of PRF. A collagen matrix

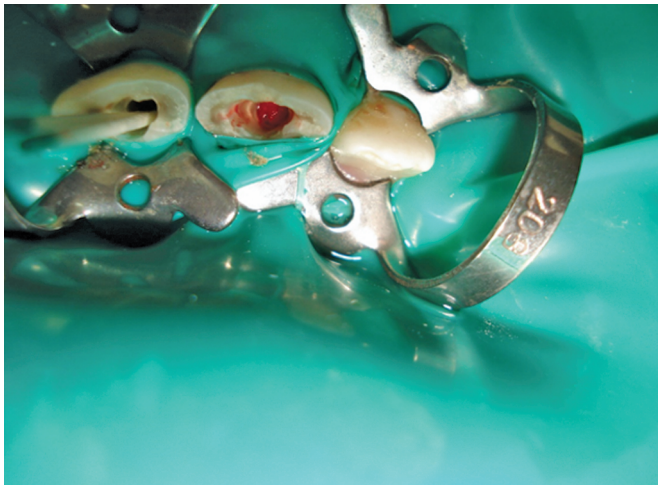


Fig. 1: Blood filled the canal till CEJ

[Collacote (Integra Life Sciences Corp., Plainsboro, NJ, USA)] was place on topmost of the BC, allow the matrix to soak with blood to circumvent development of a chamber. Mineral trioxide aggregate was placed using Amalgam carrier on Collacote.

PRF Scaffold (Examined Group)

Blood Collection: About 5 mL of intravenous blood from the antecubital vein was collected in a sterile plastic syringe without anticoagulant. Blood from the sterile plastic syringe was transferred to 5 mL sterile glass tube then recapped to be centrifuged.

Preparation of PRF[23]

Centrifugation machine (i Fuge D06 Bench Top Doctor Centrifuge, Neuation Tech Pvt. Ltd, India) was balanced by adding saline tube opposite to blood containing tube. Collected blood was centrifuged immediately at 2,400 rpm for 12 minutes to obtain the PRF. The PRF was cut into small portion using a sterile scalpel blade then introduced inside root canal and pushed toward beyond apical region using endodontic pluggers till the level of the cementoenamel junction. Collacote was seated above PRF. Then, an MTA was layered covered by a moist cotton pellet then glass ionomer. The patient was recalled after 3 days to confirm the setting of MTA then access cavity is sealed with a layer of glass ionomer material and composite.

Posttreatment Evaluation

Treated teeth were evaluated at 3, 6, 9, and 12 months. Primary outcomes measured were sinus/fistula formation, pain complaint, mobility grade, and swelling presence/absence. Radiographic: root length elongation and increase in root thickness. Secondary outcomes were sensibility test and crown color change. Radiographic: change in bone density and apical diameter (Fig. 2).

Image Analysis

Changes detected were calculated using Image J software by the same method described by Nagy et al.[24]

Statistical analysis was accomplished using SPSS statistical version 19 using Wilcoxon signed-rank test and the *t* test for dependent samples when the population cannot be assumed to be normally distributed, and the size of the sample is <30. The Mann–Whitney *U* test is used to test whether two samples are likely to derive from the same population. The level of significance is set to be $p < 0.05$.

Results

Flowchart 1 shows the stream of the participants through the study.

Demographic Data

Table 1 designates the demographic variables of the patients in each group.

Primary Clinical Outcomes

No statistically significant difference concerning primary clinical outcomes between the two groups were established with all treated teeth showed 100% success.

Primary Radiographic Outcomes

Tables 2 and 3 show the primary radiographic outcomes (root thickness and length).

Secondary Clinical Outcomes

All treated teeth were negative to sensibility test. Blood clot displayed greater crown discoloration in comparison to PRF group with no statistical significance difference between them.

Secondary Radiographic Outcomes

Tables 4 and 5 show the secondary radiographic outcomes.

Discussion

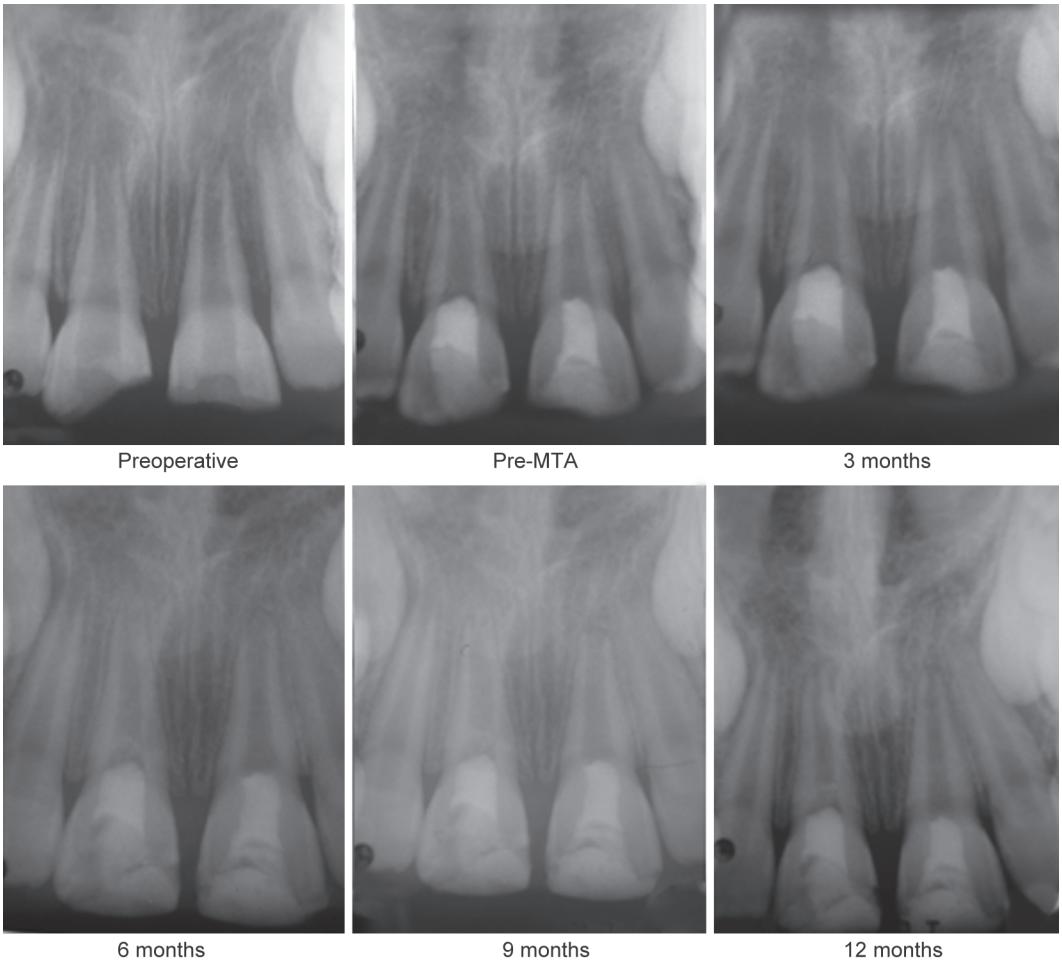
The split-mouth study formulation is an illustration of a randomization on spot level where control and intervention groups are randomly categorized to sites of one of the two halves of the mouth. The charm of the split-mouth is the deduction of much of the intersubject variability by this means enhancing the power of the study compared to the whole-mouth design. In other words, it aims to remove all elements related to differences between patients, by making within-patient comparisons, rather than between-patient comparisons thus reducing the sample size.[25]

"All RCTs assess response variables, or outcomes (end points), for which the groups are compared. Most trials have several outcomes, some of which are of more interest than others. The primary outcome measure is the pre-specified outcome considered to be of greatest importance to relevant stakeholders (such a patients, policy makers or clinicians)" as stated by CONSORT statement 2010.[26,27] In this study, the primary and secondary outcomes were assessed.

In the present study, treatment success was demarcated as the removal of symptoms, the resolution of apical radiolucency with root increase in length and thickness, and a decrease of the apical foramen. Discoloration and root calcification were not considered failure as it is inevitable in pulp regeneration cases.[11,28]

Cases treated showed no failure in treated groups. Similar findings were depicted by Shivashankar et al.[29] and Bakhtiar et al.[16]

Platelet-rich fibrin showed statistical significance increase in root length either in millimeter or percentage than that of BC group for all time points. This finding was in consistent with the results obtained by Narang et al.,[30] Prabhakar et al.,[31] and Sharma and Mittal.[32] Platelet-rich fibrin displayed statistical insignificance increase in root dentin thickness in either millimeter or percentage when compared with BC group for all time points, this was in agreement with Zhou et al.[33] This could be explained



Figs 2A to F: Group I (BC) vs group II PRF: Immature bilateral, fractured teeth (#11 and 21) with an open apex in an 8-year-old girl. Eleven was treated with PRF and 21 was treated with blood clot: (A) Preoperative Periapical radiograph; (B) After the placement of mineral trioxide aggregate (MTA); (C) At the 3-month follow-up; (D) At the 6-month follow-up, with continued development of the root; (E) At the 9-month follow-up, with continued development of the root apex; (F) At 12-month follow-up, complete maturation of the root apex with hard tissue bridge formation noted on cervical 1/3 of root in 11

by PRF being improved origin of signaling molecules compared to BC, that have been used for augmenting the regeneration defective dentin and the dentin–pulp tissues. Platelets are rich in cytokines and signaling molecules, differently involved in tissue regeneration, and have a vital role on cellular differentiation. Platelet-rich fibrin contains thrombin that is capable of creating equal-sided junctions in polymerized fibrin, with resultant sustained signaling molecules discharge and a moldable fibrin network, which is a proper microenvironment for cell migration. All which composing PRF is a preferred scaffold in RET producing better tissue healing.[32,34]

Pulp sensibility tests for teeth in both groups showed negative response during follow-up period. These results comply with Petrino et al.,[7] other studies reported variable positive responses to pulp sensibility tests.[14,35]

Variability of the results could be attributed to long-standing periapical disease in cases with periapical radiolucency which have retarded neural regeneration. Negative results to pulp sensibility test could be due to coronally present MTA layer which act as insulator, or the need of >12 months for complete formation of blood vessels and nerve fibers within the root canal.[36]

As demonstrated by histological studies, increase thickness of the root canal walls is largely caused by deposition of cementum-like tissue with non-tubular pattern seen in dentin.[37,38]

For teeth discoloration, the percentage of teeth displayed discoloration for BC group is greater and statistically not significant than in PRF group for all time points. The results were in line with Nagata et al.[39] and McTigue et al.[40] Scaffold may play a role in discoloration by interacting with MTA during its setting. Blood by itself could lead to discoloration by the accretion of hemoglobin in dentin. Staining of MTA is visible when material sets in interaction with red blood cells (RBCs).[41] It could be hypothesized that iron ions and development of calcium alumino ferrate play a role in discoloration. Furthermore, porosities that might present in MTA which can absorb blood components.[42] All calcium silicate cements possess high susceptibility to discoloration in contact with blood. The probable method to prevention discoloration is the use scaffold without RBCs, such as PRF.[42]

One PRF treated teeth showed signs of canal calcification/obliteration. While three teeth from the BC scaffold showed obliteration of some of the canal spaces. Undesirable outcomes in revascularization/revitalization is partial or complete obliteration of

Flowchart 1: CONSORT flow diagram presenting the flow of the patients through the study

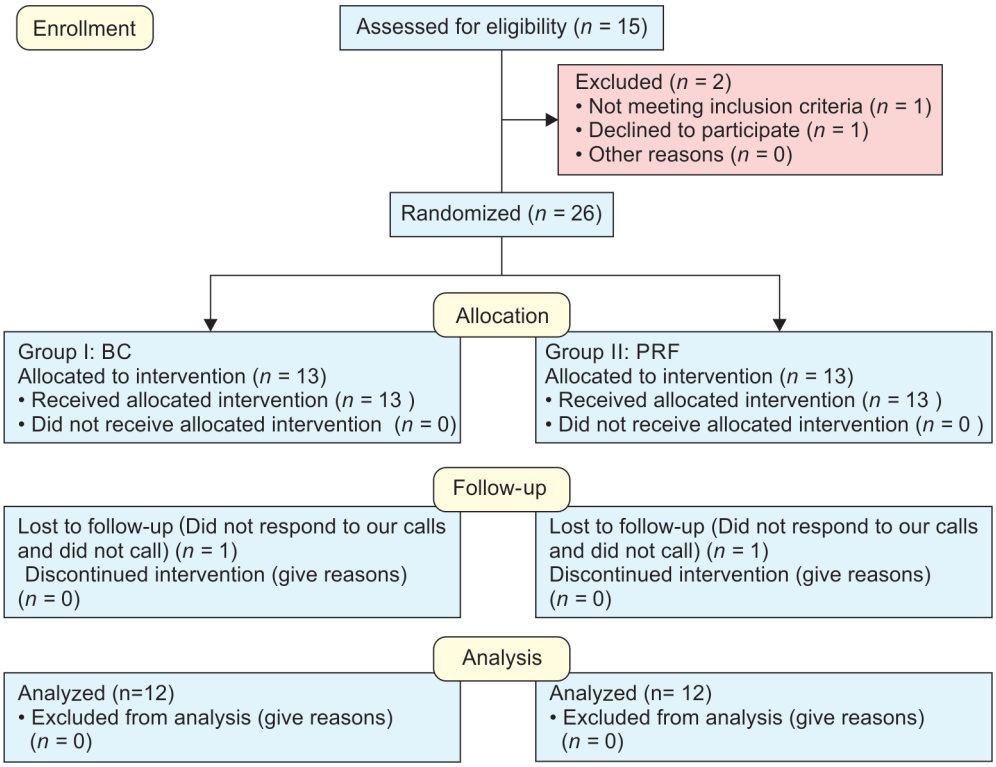


Table 1: Sample description according to the basic characteristics of the patients

Variable	*BC vs PRF (n = 12), n (%)*		*p value*
Age			
Mean ± SD	9.08 ± 1.165		0.989
Sex			
Male	6 (50%)		0.582
Female	6 (50%)		
Type of trauma	Right	Left	
Enamel–dentin–pulp fracture	11 (91.7%)	1 (8.3%)	0.013**
Enamel–dentin	1 (8.3%)	11 (91.7%)	0.013**
No loss of tooth structure	0 (0%)	0 (0%)	

*Significant at *p* value <0.1
**Highly significant at *p* value <0.05

Table 2: The increase in root length in millimeters and percentage of the PRF vs BC groups in the four evaluation periods

	PRF group (mean ± SD)	*BC group (mean ± SD)*	*p value*
3 months (mm, %)	0.155 ± 0.099 (1.02% ± 0.673%)	0.104 ± 0.081 (0.668% ± 0.507%)	0.158
6 months (mm, %)	0.391 ± 0.187 (2.57% ± 1.23%)	0.193 ± 0.08 (1.24% ± 0.495%)	0.01**
9 months (mm, %)	0.793 ± 0.378 (5.2% ± 2.48%)	0.376 ± 0.162 (2.42% ± 1.01%)	0.005**
12 months (mm, %)	1.24 ± 0.54 (8.19% ± 3.64%)	0.608 ± 0.228 (3.93% ± 1.46%)	0.005**

*Significant at *p* value < 0.1
**Highly significant at *p* value < 0.05

the pulp canal, with the precise mechanism for the calcification is unknown.[43] Osteoinductive capacity of MTA could be the cause.[44] Canal calcification might be a factor in crown discoloration. In addition, it might create difficulties for future root canal treatment if required.

The study showed increase bone density by time in both groups. However, the mean of the increase of bone density in the PRF group is greater than that of BC group for all time points. This difference is statistically significant. This finding is in consistent with Prabhakar et al.[31] and Sharma and Mittal.[32] These results could be explained by

Table 3: The increase in root thickness in millimeters and percentage in the four evaluation periods

	PRF group (mean ± SD)	*BC group (mean ± SD)*	*p value*
3 months (mm, %)	0.19494 ± 0.172 (7.9% ± 6.2%)	0.133 ± 0.127 (6.3% ± 7.6%)	0.272
6 months (mm, %)	0.474 ± 0.299 (19.97% ± 12.08%)	0.377 ± 0.332 (17.65% ± 18.97%)	0.346
9 months (mm, %)	0.73517 ± 0.34 (30.77% ± 13.26%)	0.58 ± 042 (27.6% ± 28.37%)	0.182
12 months (mm, %)	0.903 ± 0.392 (39.37% ± 16.49%)	0.74 ± 0.54 (39.07% ± 35.22%)	0.117

*Significant at *p* value <0.1
**Highly significant at *p* value <0.05

Table 4: The increase in bone density in gray value and percentage of PRF and BC groups in the four evaluation periods

	PRF group (mean ± SD)	*BC group (mean ± SD)*	*p value*
3 months (gray value, %)	15.64 ± 11.1 (45.14% ± 74.78%)	13.5 ± 9.88 (0.265% ± 0.34%)	0.012**
6 months (gray value, %)	28.2 ± 16.7 (80.6% ± 145.1%)	28.21 ± 19.8 (0.599% ± 0.97%)	0.012**
9 months (gray value, %)	40.79 ± 19.12 (109.29% ± 171.22%)	45.98 ± 24.1 (0.88% ± 1.07%)	0.012**
12 months (gray value, %)	53.44 ± 22.165 (137.4% ± 203.02%)	59.08 ± 27.38 (1.07% ± 1.2%)	0.012**

*Significant at *p* value <0.1
**Highly significant at *p* value <0.05
SD, standard deviation

Table 5: The decrease in apical diameter in millimeters and percentage of the PRF and BC groups in the four evaluation periods

	PRF group (mean ± SD)	*BC group (mean ± SD)*	*p value*
3 months (mm, %)	0.34 ± 0.2 (15.7% ± 8.84%)	0.274 ± 0.163 (11.98% ± 6.8%)	0.084**
6 months (mm, %)	0.87 ± 0.48 (38.23% ± 15.03%)	0.77 ± 0.5 (32.68% ± 13.82%)	0.021**
9 months (mm, %)	1.33 ± 0.57 (58.89% ± 10.59%)	1.15 ± 0.52 (50.7% ± 10.93%)	0.004**
12 months (mm, %)	1.73 ± 0.665 (76.75% ± 8.5%)	1.47 ± 0.63 (64.6% ± 11.6%)	0.006**

*Significant at *p* value < 0.1
**Highly significant at *p* value <0.05
SD, standard deviation

constant release of signaling molecules, improving the enrolment, retention, and proliferation of undifferentiated mesenchymal and endothelial cells from the periapical area. It encourages collagen production. It also outputs anti-inflammatory agents (ANTES/CCL5) (regulated upon activation, normal T-cell expressed, and secreted, a protein classified as a chemotactic cytokine or chemokine) that controls the local inflammatory response and improves soft- and hard-tissue wound healing.[45]

The decrease in apical diameter in the PRF is greater than that of BC group for all time points. This difference is statistically significant. This is in agreement with Prabhakar et al.[31] and Sharma and Mittal.[32] Platelet-rich fibrin functional theory is stimulating stem cell proliferation and higher expression of osteoprotegerin proteins and alkaline phosphatase. These proteins are usually identified as odontoblast differentiation markers.[46]

Conclusion

For necrotic permanent teeth, with open apex RET using PRF is a desirable substitute to BC and shows excellent 12-month prognosis. Though PRF gives better results compared to BC, it requires blood withdrawal from the child which might be difficult in needle phobic children.

Recommendations

- Regenerative endodontic techniques should be the prime treatment in children with necrotic permanent teeth with open apex as it displays excellent results.
- While deciding to perform regenerative endodontic procedure, PRF should be the target treatment as scaffolding material when compared with BC.

Limitations

- Pulp blood supply should have been assessed which was performed by laser Doppler flowmetry and pulse oximeter.
- Histological assessment of intracanal formed tissue could not be assessed due to ethical reasons.

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