Comparative Study between Revitalization of Necrotic Immature Permanent Anterior Teeth with and without Platelet Rich Fibrin: A Randomized Controlled Trial

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Objectives: *This study was conducted to evaluate the effect of platelet rich fibrin (PRF) during revitalization of necrotic immature permanent anterior teeth after 6 months and 1 year follow up period. The following treatment protocols; Group A: revitalization using blood clot scaffold only, and Group B: revitalization using blood clot and PRF.* ***Study design:*** *twenty-two patients (7–12 years old) suffering from immature necrotic permanent maxillary central incisors were randomly selected and randomly allocated into 2 groups. Clinical examinations were performed to detect any pain or swelling during the follow up period. Standardized radiographs were digitally evaluated for changes in root length, periapical radiolucency size, presence of apical or cervical calcific bridges.* ***Results:*** *After a follow-up period of 12 months, most of the cases showed radiographic evidence of periapical healing and showed calcific bridges either cervical and /or apical. No significant differences were shown between both groups.* ***Conclusions:*** *The revitalization procedures succeeded to show continued development of roots in teeth with necrotic pulps. The use of PRF was not essential for repair but it helped during the procedures.*

Keywords: *Platelet rich fibrin, regeneration, immature permanent teeth.*

INTRODUCTION

One of the worst scenarios in children dentition is a necrotic pulp of a young permanent tooth. Saving immature teeth is a crucial step in preserving the alveolar bone of a growing child and keeping his smile. Losing the pulp before completion of root formation will lead to the following cons; short root, wide apex and thin root walls. An endodontic root canal treatment is required to maintain this tooth; first, controlling the infection, second; creating an apical tight seal. These aims offered by calcium hydroxide apexification or by creating an instant apical barrier using mineral trioxide aggregate (MTA). But a problem still exists which is a short root with thin root walls; liable to future fractures. A paradigm shift for treatment of such cases emerges by the development of tissue engineering technologies using stem cells; regeneration or commonly called revitalization, revascularization or maturogenesis [1]. Revitalization changes the necrotic root into a vital root able to complete its development. Regeneration not only closes the wide apex as that by the apexification technique but also increases root wall thickness and root length [2]. A stronger root with an adequate crown-root ratio is obtained; which is more resistant to future fracture.

Revitalization of non-vital teeth allows repair and regeneration of tissues. The rationale of revitalization is that pulp vitality can be reestablished if a sterile tissue matrix is provided in which new cells can grow. Components needed for tissue engineering; stem cells, signaling molecules and scaffold. The first case reports for revitalizing a necrotic pulp were published in 2001[1]. Platelet-Concentrates are recently used as a physical scaffold in revitalization of necrotic immature teeth [3]. There are two generations; the first generation; platelet rich plasma (PRP) and the second generation; platelet rich fibrin (PRF). PRF and PRP can be added to enrich the natural blood clot [2]. The scientific rationale behind the use of platelet preparations lies in the fact that the platelet alpha granules are a reservoir of many growth factors that are known to play a crucial role in hard and soft tissue repair mechanism.

PRF was developed in France by Choukroun *et al*, in 2001. PRF can be considered as an autologous biomaterial which consists of

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leukocytes, platelets, and range of healing proteins within dense fibrin matrix [3]. This material can work as reservoir for active biochemicals which are released slowly and continuously over a period of 7–14 days [3,4]. PRF enhances the proliferation of various cell types, stimulates cellular differentiation and supplements the angiogenesis. Also, the presence of leucocytes and cytokines along with small amounts of lymphocytes in PRF can play a significant role in the self- regulation of inflammatory and infectious phenomenon [4]. The aim of this study to evaluate the effect of platelet rich fibrin (PRF) during revitalization of necrotic immature permanent anterior teeth after 6 months and 1 year follow up period.

MATERIALS AND METHOD

A Parallel Randomized Controlled Trial (RCT), Double blinded (patients and assessors were blinded) were designed. Ethics approval by the Human Research Ethics committee at Faculty of Dentistry, Cairo University was obtained. The trial was registered in PACTR Registry; www.pactr.org, its number is: PACTR201304000530301.

Sample size calculation was done using PS program. We planned a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. The proposed standard deviation is 1.2 mm as calculated by [5], we will need to study 11 experimental subjects and 11 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

Patients were selected from the outpatient clinic of Pediatric and Community Dentistry Department, Faculty of Dentistry, Cairo University. A total of twenty-two necrotic immature maxillary anterior permanent teeth in twenty-two subjects were included as satisfying the eligibility criteria. 15 males & 7 females aged between 7-12 years old with mean age 9.86+/-1.55 years.

Inclusion criteria

1. Traumatized permanent anterior teeth showing signs/ symptoms of pulp necrosis; apical periodontitis, abscess, fistula or discoloration**.**
2. Preoperative radiographic assessment confirming that the radiographic apex is more than 1mm in diameter.
3. Probing depth less than 3 mm
4. Restorable teeth
5. Positive patient's acceptance for participation in the study.

Exclusion criteria

1. Subjects who have any medical, physical or mental handicapping condition.
2. Pathological tooth mobility.
3. Radiographic evidence of external or internal root resorption.
4. Any known sensitivity or adverse reactions to the antibiotics in the bi-mix (ciprofloxacin and metronidazole).

Parent/patient guardians written approval were taken by signing the informed consent after discussing the treatment plan, all the possible outcomes and the anticipated prognosis. Each participant was randomly assigned following simple randomization procedure by using closed opaque white envelops (22 closed white envelopes) which assign the group to be followed. Those closed envelops includes 11 paper charts written in it "WITHOUT PRF" and 11 paper charts written in it "WITH PRF"**,** which were folded three times not to show its contents to assure random assignment.

Root Canal Treatment Procedures

First Dental Visit

The target of the first dental visit for all the patients was to control the infection and overcome all the signs and symptoms.

1. Rubber Dam isolation using Optra Dam Plus, Size Small *(OptraDam Plus, Size Small* (IvoclarVivadent)).*
2. Access preparation using round bur and stone.
3. The canal was then irrigated with 20 ml of 5% sodium hypochlorite for 10 minutes [6], followed by saline irrigation.
4. Canal dryness was done by paper points.
5. Antibiotic paste was placed inside the canal using amalgam carrier and suitable condenser. The canal was filled to a level just below the cemento-enamel junction (CEJ). Two antibiotics were used as that used by[1].

   One tablet from metronidazole (500mg) *(Flagyl 500 mg tablets, Sanofi Aventis)* and one tablet from ciprofloxacin (500 mg) *(500 mg tablets, European Egyptian Pharm. Ind.)* were grinded to form a homogenous powder which was mixed with distilled water to form a paste of reasonable consistency
6. The access cavity is sealed with small pellet of cotton and glass ionomer cement *(KetacCemEasymix, 3M Deutschland GmbH, Germany)* to ensure a coronal seal until the next visit. After 3 weeks, If a complete resolution of signs and symptoms; pain, swelling and fistula were confirmed then the second dental visit was done.

Second Dental Visit

Grouping of subjects according to the usage of PRF into 2 groups allowing the child to withdraw a white envelope previously mentioned to locate him randomly in either group: Group A: Eleven teeth were revitalized without using PRF, Group B: Eleven teeth were revitalized using PRF.

Group A: Without Using PRF

1. The Patient was anesthetized using plain anesthesia 3% mepivacaine *(Alexandria Co. for Pharmaceuticals, Alexandria, Egypt)*
2. Rubber dam isolation using Optra Dam.
3. Access was re-opened using round bur and the antibiotic mixture was washed out using 5% NaOCl followed by saline irrigation.
4. The canal was dried using paper points.
5. H-file *(Mani, Zhengzhou Shengxin Medical Instrument Co., Ltd., made in Germany)* size 60 was used to irritate

the apical tissue and induce bleeding to a level below CEJ. Excess blood was dried using small cotton pellet held with tweezer.

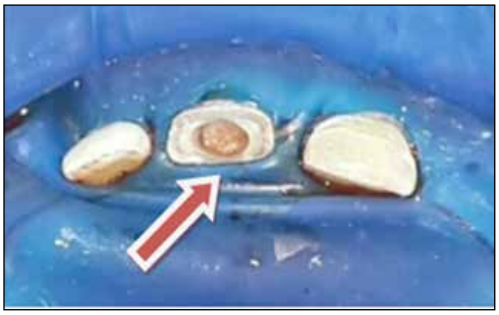
6. Over 15 minutes, the blood was allowed to clot.
7. Then 3mm of grey MTA *(Reparative cement, Angelus Indústria de ProdutosOdontológicos S/A, Brasil)* was prepared according to the manufacturer instructions and was placed with a slight pressure using an amalgam carrier and a suitable condenser.
8. The access cavity was sealed with chemical cure glass ionomer (SD I, Victoria, Australia) after waiting 45 minutes till the initial setting of MTA [7].Post-operative digital radiographic image was taken as a base line radiographic photo "zero day".

Group B: Using PRF

PRF preparation. 12ml sample of whole blood was drawn intravenously from the patient's right antecubital vein and centrifuged *(Centrifuge Model 801, made in China)* under 3000 rpm for 12 minutes to obtain the PRF. Figure (1) shows extracting the PRF from the test tube after being centrifuged. PRF was jelly like in consistency, then the same steps as that of group A were followed except step number (6) as PRF was packed inside the root canal at the level of CEJ immediately after inducing the bleeding and before blood clot formation as shown by Figure (2).

Figure 1: Extraction of PRF from the centrifuged blood

Figure 2: Packed PRF below CEJ



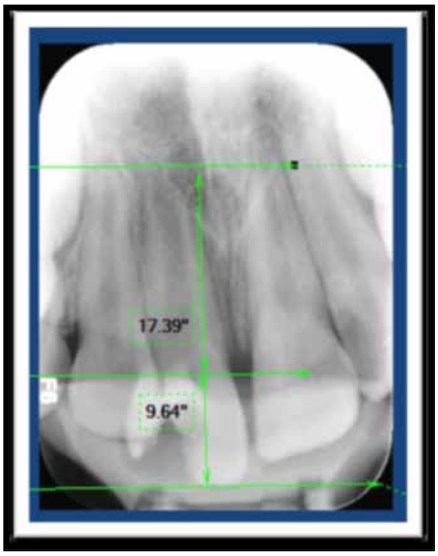
Periapical digital radiographic records were taken at zero base line record, 6 months and 1 year postoperatively. Digital images were taken using Digora system (Digora® OPTIME, Soredex Orion Corporation, Tuusula, Finland) at Oral Radiology Department, Faculty of Dentistry, Cairo University. The patients were radiographed using film sensor; photostimulable phosphor plate (PSP) (31×41 mm).

Six month to one year follow up

Radiographic Examination

1. Image Calibration: The length of the imported radiographic image to the Digora software was calibrated by 41mm as equal to the actual length measured on the used film sensor during the study**.** The calibration process permitted measurement of changes in root length to be based on a millimeter scale [5,8].
2. Lines measurements: Figure (3) shows the measuring lines to calibrate the crown root ratio. Line (a): a straight line was drawn through the CEJs of the treated tooth[8], Line (b): a straight line was drawn parallel to line (a) and tangent to the incisal edge of the tooth and Line (c): a straight line was drawn parallel to line (a) and tangent to the tooth root apex.
3. A perpendicular line was drawn from line (a) to line (b) and its length was measured to be the crown length.
4. Another perpendicular line was drawn from line (a) to line (c) and its length was measured to be the root length.
5. The crown/root ratio was measured by the same way in the three-successive digital radiographic images; base line, 6 months and 1 year follow up radiographs.
6. The readings were edited in an excel sheet for the later statistical analysis
7. All the measurements were done by two examiners; the main author and oral radiologist then repeated by the main author after 1 week period to confirm the reproducibility of the readings and to minimize the possibility of errors.
8. These readings estimated the difference in crown/root ratio in the successive follow up visits then the percentage of root length increase was calculated.
9. Although the results were generated in millimeter units, the data was presented as a ratio and percentage of change from preoperative values rather than the calculated millimeter change.. In addition, the units of percentage change provide a clinically meaningful outcome when considering the impact of the revitalization procedures [5].

Figure 3: Exemplifies the measuring lines to calibrate the crown root ratio.



Visual evaluation to the radiographic images taken through the follow up period for formation of cervical calcific bridge and apical barrier and evaluation of changes in the periapical radiolucency

Image J software was used to measure the perimeter of the periapical radiolucency through the follow up period. Image calibration was done inside the ImageJ software as that in Digora software but the ImageJ software allowed freehand selection to delineate the periapical radiolucency more accurately. The percentage of change in the perimeter of the periapical radiolucency was then calculated **(Figure 4).**

Statistical analysis

Data were statistically described in terms of mean, standard deviation, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing 2 groups when normally distributed and Mann Whitney *U* test for independent samples when not normally distributed. For comparing categorical data, Chi square (2) test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

RESULTS

Percentage of root length increase through the follow up period: figure (5) shows the percentage of root length increase through the follow up period in each group and comparing it with the other group. 1) Group A shows a percentage increase in root length 7.7% after 6 months and 14.8% after 1 year.2) Group B shows a percentage increase in root length 8.8% after 6 months and 12.3% after 1 year.3) No statistical significant difference between the two groups through the follow up period, both groups showed nearly the same rate of root length increase.

Figure 5: Bar chart representing percentage of root length increase through the follow up period in both groups.

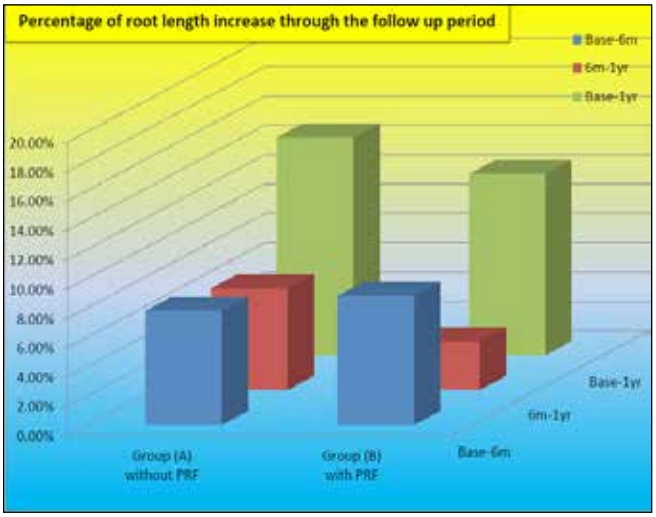


Figure 4: Example to ImageJ software while measuring the perimeter of the periapical radiolucency through the follow up period

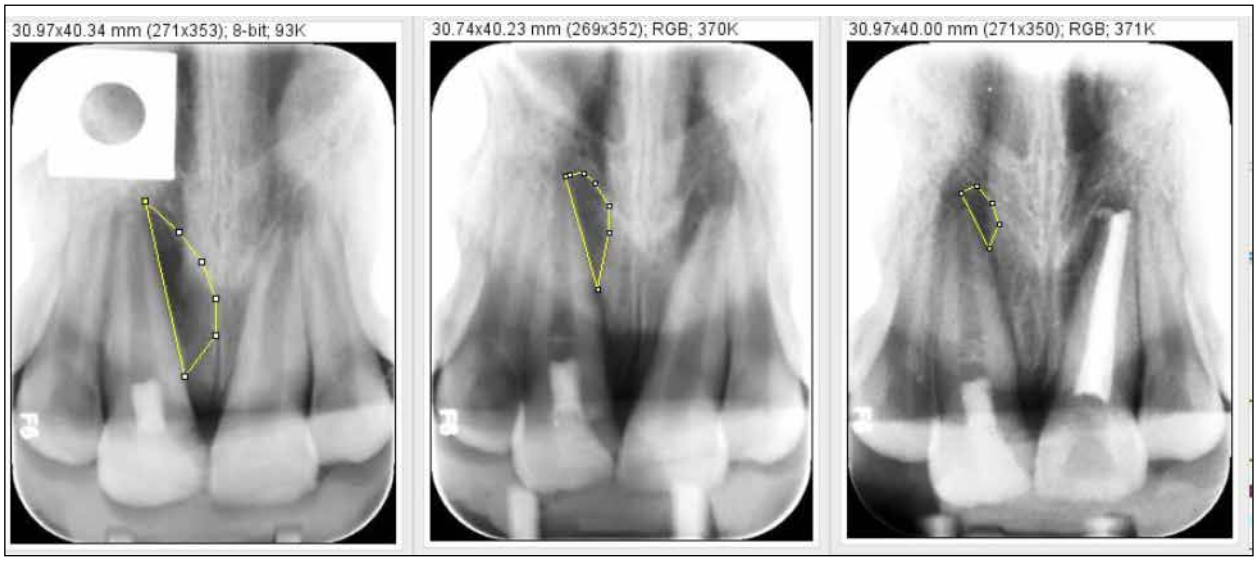


Figure (6) and figure (7) exemplifies apical and cervical calcific bridge formation post-operatively.

Table 1 summarizes the findings of calcifications at the end of the follow up period: Cervical calcific bridges were observed in 4 cases in each group. Apical barriers were observed in 5 cases in group (A) and 7 cases in group (B). · Both cervical and apical bridges were observed in the same tooth in 1 case in group (A) and 3 cases in group (B). · Total number of cases that showed calcific barriers was 8 cases out of 11 in each group. 3 cases did not show any signs of calcification and was equal in both groups representing 27% (3 cases) of each group.

Table 1: Analytical evaluation of calcific barriers at the end of the follow up period in both groups

Observed Calcific Barriers	Cervical	Apical	Both Cervical And Apical in the same tooth	Total	No Calcific barriers Observed	Total
Group (A) Without PRF	4 (36.4%)	5 (45.4%)	1 (9%)	8 (72%)	3 (27%)	11 (100%)
Group (B) With PRF	4 (36.4%)	7 (63.6%)	3 (27%)	8 (72%)	3 (27%)	11 (100%)

Figure 6: Radiographic photos showing apical calcific bridge formation; A: preoperative radiographs, B: post-operative radiographs

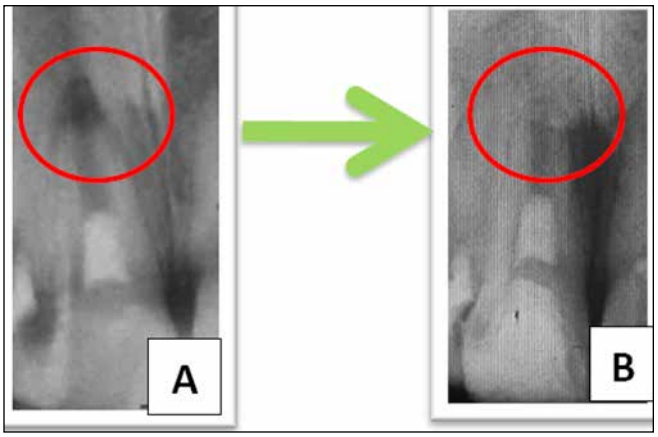
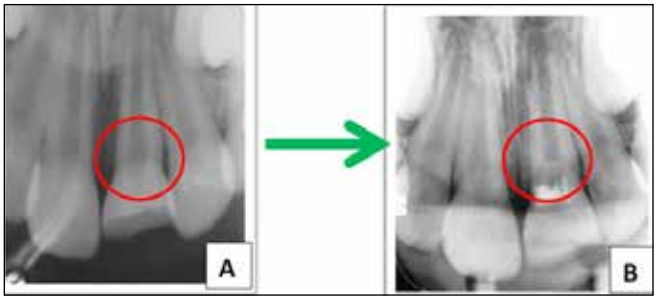


Figure 7: Radiographic evaluation of cervical calcific bridge formation; A: preoperative radiograph, B: post-operative radiograph



Evaluation of the periapical radiolucency through the follow up period

There was a decrease in the size of the periapical radiolucency through the follow up period in all the cases of both groups. Figure (8) shows the decrease in the periapical radiolucency through the follow up period. The percentages of decrease in the perimeter of the periapical radiolucency after 6 months and 1 year follow up in both groups while measuring it using the Image J software program.1) Group A shows 49.7% decrease after 6 months and 80.5% decrease after 1 year follow up. 2) Group B shows 55.4% decrease after 6 months and 74.2% decrease after 1 year follow up. 3) There was no statistical significant differences between the 2 groups through the follow up period.

DISCUSSION

The Pulpal necrosis of immature permanent teeth secondary to trauma can pose potential problems and complications that are exacerbated in a growing child [9].The present clinical trial was conducted to regenerate the traumatized necrotic immature permanent anterior teeth. Regeneration of the necrotic pulps was done using bleeding only in one group and platelet rich fibrin (PRF) in addition to the induced blood in the other group as to evaluate the PRF effect during the revitalization process.

In the current study, the mean age was 10 years. The recommended age range for regenerative endodontic therapy is from 8 to 16 years [10]. The maxillary central incisor was the tooth of interest as it was the most commonly affected tooth in dental trauma 43.8%[11].

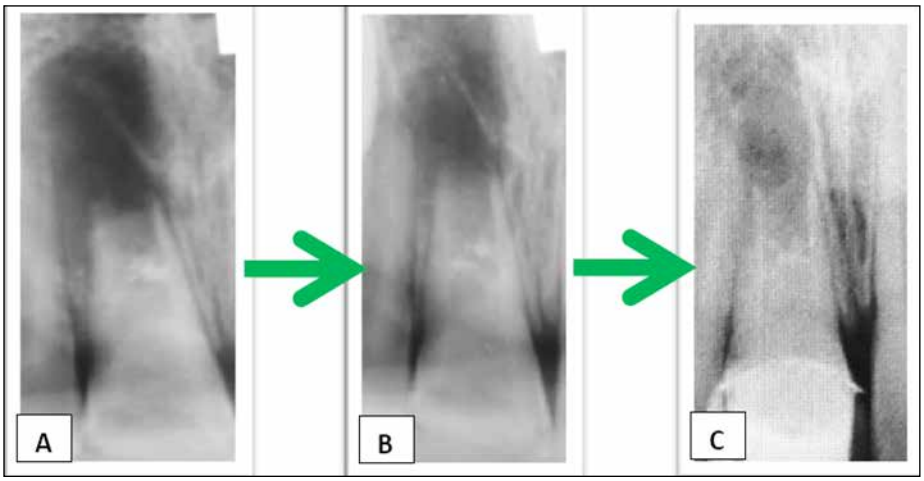
Case selection in the current study was based on the clinical and radiographic examination. The width of apical foramen should be more than 1.0 mm to allow revascularization [9]. As if the young tooth has an open apex and a short root; this allows new tissue to grow into the pulp space quickly [12].

In our study the protocol of treatment was planned to be done at two main dental visits, three weeks apart [13-15]. The first dental visit aimed to disinfect the root canal system while the second visit aimed to regenerate a pulp-like tissue inside the root canal.

The canal disinfection protocol was the same for all subjects and started at the first visit where irrigation and intracanal medicaments were used. Sodium hypochlorite (NaOCl) was the main irrigant in the current study. Concentration of NaOCl was found to be fairly suitable at 5% after reviewing the available literature. Lower concentrations are ineffective against specific microorganisms [16], while higher concentrations of NaOCl are cytotoxic to stem cells in the apical tissues [10]. NaOCl was the irrigant of choice according to several case reports[17-19].NaOCl was then flushed from the root canal with saline to reduce any lingering toxicity that can reduce the regeneration responses as recommended by [20].

To complete the disinfection protocol of the necrotic infected canals, a bi-mix of metronidazole (tablets, 500mg) and ciprofloxacin (tablets, 500mg) antibiotics was placed inside the canal. The same bi-mix was used by [1] and showed successful results. However these results disagree with [19,21-22] as they used triple antibiotic paste, which caused discoloration**.** After three weeks from the first visit; the patients were recalled for observation in accordance with [23,18,24]**.** The second visit started after complete resolutions of the signs and symptoms as its aim was to regenerate a pulp-like tissue inside a sterile canal.

Figure 8: Radiographic photos showing decrease in the periapical radiolucency through the follow up period, A: Base line radiograph, B: Follow up after 6 months and C: Follow up after 1 year



The current study exemplifies the induction of bleeding and subsequent formation of blood clot to serve as a scaffold for the periapical cells including mesenchymal stem cells which migrate into the sterile root canal and eventually induce new tissue formation within the canal space. The proposed hypothesis was to add platelet concentrates in form of PRF to evaluate its healing capabilities in the experimental group as used by [21, 14].

Washing the antibiotic paste from the canal to prepare it to receive the blood was done by using copious and gentle irrigation with 5% sodium hypochlorite followed by saline, following the same steps [10,23].

Although saline lacks antimicrobial and tissue dissolution activity, it has a good biocompatibility so used as a final rinse after irrigation with NaOCl to help promote stem cells' attachment to root canal walls in accordance with [25].

Mesenchymal stem cells are then delivered into root canal space with bleeding induction from the periapical region. These stem cells can differentiate into odontoblast-like cells forming root dentin [26]. In addition, it is assumed that the blood clot formed inside the root canal space contains platelet-derived growth factors and serves as a protein-rich scaffold [27].

The role of PRF consequents from being autologous healing biomaterial incorporating leucocytes, platelets and wide range of key healing proteins in a dense fibrin matrix [28]. It was used in the current study as a part of the main scaffold (blood clot). [14, 21] used the PRF solely as a scaffold in the canal without inducing bleeding and they showed positive results regarding the healing and root development.

Mineral trioxide aggregate (MTA) was placed directly over the blood clot or the PRF to obtain a coronal seal. MTA is not an inert dental material but is rather active in promoting hard tissue formation [29-30]. It was proved that there are leached out ions; calcium, silica, bismuth, iron, aluminum, and magnesium from MTA when come in contact with tissue fluids that result in increasing biocompatibility, sealing ability and dentinogenic activity of MTA [30].

We observed leaching out from the MTA apically in one case. This may be due to the blood was not properly clotted before the application of MTA or due to the thickness of the MTA appeared less than 3mm at the base line radiographic image. A previous study concluded that exposure of MTA to blood during setting has a negative effect on its marginal adaptation, and blood-exposed MTA has a different surface microstructure [31]. Another explanation by [32] is that MTA which set in the presence of blood has inferior physical properties, i.e., reduced compressive strength and micro-hardness and less resistance to displacement).

Glass ionomer was used to create a double coronal seal that was necessary for the treatment success. Glass ionomer was injected over the MTA at the second visit after waiting 45 minutes to allow the initial setting of MTA [7].

In the current study, all the cases considered clinically successful in both groups in accordance with [5] who stated that regenerative endodontic cases were clinically successful based on the regression of signs and symptoms associated with infected necrotic teeth.

Discoloration was clinically observed in all treated teeth and considered the main complaint by the patients. It was documented in others' cases like that in [25, 30, 24, 22]. Although minocycline was excluded to avoid discoloration, but MTA proved to be a main cause for the induced discoloration due to its iron and magnesium contents [33, 30.]

Radiographic assessments were done to evaluate the percentages of root length increase, the differences in the crown root ratios, the healing of the periapical radiolucencies and to monitor formation calcific barriers. Each case is normalized to its own base line records at the zero date (the date of the second visit) measurement as that done by [5].

Although it is difficult or impossible to obtain a perfectly standardized radiographic image [34], a standardized radiographic protocol was followed to obtain minimal radiographic geometric error.. As we found differences in the crown length in the radiographic images although it is a stable object in the three successive films. The differences were minimal; example; a crown length was measured to be 7.09, 7.01 and 7.03 in the three radiographic images (base line, after 6 months and after 1 year). But that could affect the results while measuring the minute root length increase through the one year follow up period. To overcome this radiographic error; the crown-root ratio was measured as any error will be cancelled in the ratio.

Radiographic analyses showed there is a decrease in the crown root ratios through the follow up period in both groups. This decrease meant that the root length increased. The same data for the crown and root measurements through the follow up period were mathematically used to calculate the percentage of root length increase.

The percentage change calculations provide a clinically meaningful outcome when considering the impact of regenerative endodontic procedures [5].

In the current study the percentage of root length increase after 1 year were 14.8% in group A and 12.3% in group B. These percentages were comparable with others' outcomes. The radiographic outcomes of the percentage increase in root length for revascularization was 14.9% in the retrospective study done in Mahidol University [8].

The current study lacks analysis to the changes in root wall thickness. As we found the follow up period for one year was too short to produce a measurable change in the root walls thickness. We substituted this defect by evaluating the presence of apical calcification, as even a blunt root apex might be considered as apical closure [35]. The results showed that apical calcific barriers were observed in **45.4%** of group A while in **63.6%** of group B at the end of the follow up period.

The periapical healing in terms of decrease in size of the periapical radiolucency were clearly noticed while assessing the three radiographic films visually. All the treated teeth in both groups showed gradual obvious decrease in the periapical radiolucency throughout the follow up period, there was no dropout. The percentage of decrease was measured by using the free online ImageJ software (ImageJ v1.44; US National Institutes of Health, Bethesda, MD) to obtain a quantitative data. The free hand and polygon selections in the ImageJ software were used to delineate the periapical radiolucency and thus the perimeter of each was instantly calculated by the software. The results showed the percentage of decrease in the periapical radiolucency were **80.5%** in group A and **74.2%** in group B. This decrease reflects the healing which is considered a success in the followed revitalization procedures.

During the radiographic assessment; a cervical calcific bridge was observed at the 6 month follow up. It was observed in 36.4% in each group (five cases out of eleven in each group). We considered the formation of cervical calcific bridge a good indicator for presence of vital tissue inside the previously necrotic canals that was able to react and precipitate calcific bridges. [36] recorded the presence of a hard tissue bridge under the MTA in the coronal portion of the root. [34] evaluated the responses of immature teeth to revitalization therapy and mentioned that it is not known why a hard tissue barrier was formed in the canal space between the coronal MTA plug and the root apex in two cases out of twenty in their case series. We agreed with the explanation of [10] who discussed its formation which may be due to the good biocompatibility and less cytotoxicity of MTA which allow cell proliferation and cell attachment.

The follow up period in the current study was conducted up to one year. The clinical evaluation was satisfactory to this time period. The radiographic evaluation after one year follow up was quiet enough to show resolution of the periapical radiolucency and minimal changes in root length while it was not enough to record changes in root dentinal wall thickness. That was supported by[10] recommendations who mentioned that a 12 to 18 month recall was probably the minimal time to judge radiographic evidence of root development and to conduct the clinical examination.

Based on our clinical and radiographic examinations we can say with certainty that the pulp space had returned to a vital state whatever the type of tissue formed. The healing of the apical bone and resolution of symptoms even in the absence of continued root growth may be an acceptable outcome. Survival, or retention of the treated tooth, is an accepted outcome measure. Loss of a permanent tooth before completion of alveolar bone development can lead to atrophy of the alveolar ridge, thus potentially compromising future implant replacement. A reasonable goal of revitalization procedures could be retention of the treated tooth until completion of alveolar ridge development[26]. Although limited in its extent the results of the current study suggest that PRF may not be essential for revitalization of necrotic immature permanent anterior teeth. Additional clinical studies are needed.

CONCLUSIONS

1. Controlling the infection was the corner stone for the treatment success.
2. Ciprofloxacin and metronidazole in addition to NaOCl were quite effective in controlling the infection although an extended period was required in some cases.
3. Blood clot was important to create a vital tissue inside the sterile empty canals.
4. Platelet rich fibrin was useful for the controlled placement of MTA to the desired and optimal level, with only light pressure placed on the MTA during packing.
5. PRF may not be essential for revitalization of necrotic immature permanent anterior teeth.
6. Although clinically all the cases showed complete resolution of the signs and symptoms but the radiographic outcomes varied greatly.

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