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Evaluation of Platelet-Rich Fibrin (PRF) as A Scaffold in Regeneration of Pulp in Young Permanent Anterior Teeth

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ABSTRACT

Aim: The present study was designed to evaluate platelet-rich fibrin (PRF) as a scaffold in regeneration of pulp in young permanent anterior teeth. **Subjects and Methods:** The revascularization procedure was performed in 16 cases of necrotic immature permanent teeth in all the 16 cases, the intracanal disinfection was performed with limited mechanical instrumentation, abundant irrigation, and calcium hydroxide. At the final appointment, intracanal bleeding was induced. In one group PRF will be obtained by centrifuging the blood withdrawn from the patients, well placed with a plugger into the root canal until it reaches up to the coronal 1/3rd level of the canal, in the second group laceration of the periapical area will be done to induce bleeding then MTA will be used to direct contact with the tissue then cavity restored with glass ionomer cement.Clinical and radiographic follow up will be done at 3,6,9and 12 months intervals. **Results:** After the follow-up, it was observed that the first group (PRF) had better radiographic results compared to the second group (blood clots). The first group also showed excellent periapical healing, root completion, and thickening of root canal walls. However, the second group experienced delays in the regeneration process. **Conclusion:** Regenerative endodontic treatment may be a good alternative for the treatment of young permanent teeth instead of traditional methods that result in a closed apex without any root development.

KEYWORDS

Young permanent, platelet-rich fibrin, revascularization, regenerative endodontic

INTRODUCTION

Pulpitis, necrosis, and apical periodontitis can develop after an injury to an immature permanent tooth (IPT), which can hinder root development. Conventional endodontic and periapical procedures cannot revive the tooth's necrotic pulp and promote root growth. Because of this, patients are more likely to lose teeth when IPTs with thin dentinal walls fracture[1,2,3]. Dentists are becoming more and more interested in pulp regeneration procedures (REP) to activate alternative pulp to continue root growth and strengthen the dentin wall in an effort to treat IPT and to stop additional tooth loss. However, there is debate over REP's efficacy and the precise techniques for implementing it.[4,5]

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For developing teeth with necrotic pulp, a biologically based alternative therapy called regenerative endodontic therapy has been developed.[6] Procedures that preserve the mesenchymal stem cells of the apical papilla and the remaining dental pulp stem cells can lead to continuous root development and intracanal revascularization. [7]

The success of tissue engineering depends on three factors, according to Hargreaves et al. [8] They consist of stem cells with the capacity to form hard tissues, signaling molecules that promote cellular proliferation, differentiation, and stimulation, and lastly, a three-dimensional physical scaffold that can facilitate cell growth and differentiation.

Scaffolds are among the most crucial elements of tissue engineering. Scaffolds offer assistance with cell vascularization, differentiation, proliferation, and organization. The ideal scaffold should have the following characteristics be porous (to allow for the placement of cells and growth factors), be biocompatible with the host tissues, have the proper shape and form to allow for the replacement of the lost tissues, and be biodegradable with no toxic byproducts.[9] Dental pulp cells (DPCs) have been used in conjunction with a variety of biodegradable or permanent natural (blood clot, platelet-rich fibrin (PRF), hyaluronic acid, chitosan, and chitin) or synthetic (polylactic acid, polyglycolic acid, tricalcium phosphate, and hydroxyapatite) scaffolds to regenerate dentin or dentin-pulp complexes. [10] The benefits of natural scaffolds include good biocompatibility and have better mechanical properties and greater control over the rate of degradation as well as bioactivity. [9]

Platelet-rich fibrin (PRF), which is obtained by removing the middle layer from a centrifuged blood sample, is one such biologically based substance. Choukroun et al. provided the first description of PRF, which is also known as a second-generation platelet concentrate. It has been demonstrated to have several benefits over conventionally prepared platelet-rich plasma (PRP). Its main benefits include simplicity of preparation and the absence of blood biochemical handling, making this preparation strictly autologous. [11]

The purpose of this study was to present the clinical and radiological outcomes following the application of regenerative endodontic therapy to anterior teeth with necrotic pulp and an open apex. A typical blood clot or a PRF served as the scaffold in each group.

SUBJECTS AND METHODS

This study was carried out in the Department of Pediatric Dentistry, Faculty of Dentistry, Tanta University and it started after the institute's ethical committee issued a certificate of no clearance. We enlisted 16 child patients, both sexes, 9 to 12 years old, with immature permanent teeth that are dead. Patients with systemic illnesses that can lead to delayed healing or bleeding disorders were excluded. All of the parents of the patients who had been enrolled in the study were informed about it in everyday terms, and the study only began after they had signed a consent form indicating their willingness to take part. The case number of cases was determined using the following criteria: significance level of 0.05, power of 80%, and number of clusters (immature teeth) of 24.

Patients with necrotic immature permanent maxillary incisors with open apex, with or without radiographic evidence of periapical lesion, were included in the study, while medically compromised patients with systemic disorders that can lead to delayed healing or bleeding disorders were excluded.

A rubber dam was placed to isolate the tooth before creating a tooth access opening using a #2 round diamond bur (Endo Access Bur, DENTSPLY Maillefer) after administering local anesthesia.

Additionally, axial wall extensions were made by using a safe tip fissure carbide bur (Endo-Z Bur, DENTSPLY Maillefer). The working length was determined using periapical x-ray film. The canals

were then cleaned with minimal instrumentation to remove necrotic tissue while also preventing further weakening of the lateral dentinal walls. A 20 mL solution of 1.5% sodium hypochlorite (Prevasol U.A.E) was used to thoroughly irrigate the canals, followed by 20 mL of saline using a side-vented needle. To ensure complete disinfection of the root canals, a triple antibiotic paste was introduced into the canals until it reached about the cementoenamel junction. This paste was used as an interappointment medicament. After that, the access cavity was sealed with the temporary sealing material Cavite (3M Germany) for 4 weeks. [12]

Triple Antibiotic paste (TAP)

TAP offers significant advantages in endodontic treatment, including broad-spectrum antimicrobial activity, the ability to penetrate dentinal tubules, reduction of bacterial load, addressing antibiotic-resistant strains, and anti-inflammatory properties. It was prepared from Metronidazole (400mg), Ciprofloxacin (500mg), and Clindamycin (50mg) with a ratio of 1:1:1 were mixed and then combined with propylene glycol to create a creamy mixture of TAP. [13,14]

During the second visit, after four weeks of being free from clinical signs and symptoms, the teeth were anesthetized and isolated with a rubber dam to access them again. The canals were flushed with saline to remove the triple antibiotic paste. Then, the canals were irrigated with 17% Ethylene Diamine Tetra Acidic Acid (EDTA) and dried with paper points.

The cases were randomly split into two groups based on the method used for revascularization.

Group I used platelet-rich fibrin (PRF).

Group II used both blood clot and Mineral Trioxide Aggregate (MTA).

A standard protocol for preparing Platelet-rich fibrin (PRF) has been outlined by Choukroun et al. [15] To prepare PRF, 5 milliliters of whole vein blood is drawn into 10 ml tubes containing anticoagulant. The tubes are then immediately centrifuged at 3000 rpm for 10 minutes. During the centrifugation process, the blood comes in contact with the test tube wall, which activates the platelets and initiates the coagulation cascade. The final product consists of three layers (as shown in Figure 1): the PRF clot, platelet-poor plasma, and red blood cells (RBCs). The fibrin clot is formed in the middle of the tube, with RBCs at the bottom and acellular plasma at the top due to the conversion of fibrinogen into fibrin by the circulating thrombin, which is concentrated on the higher part of the tube.

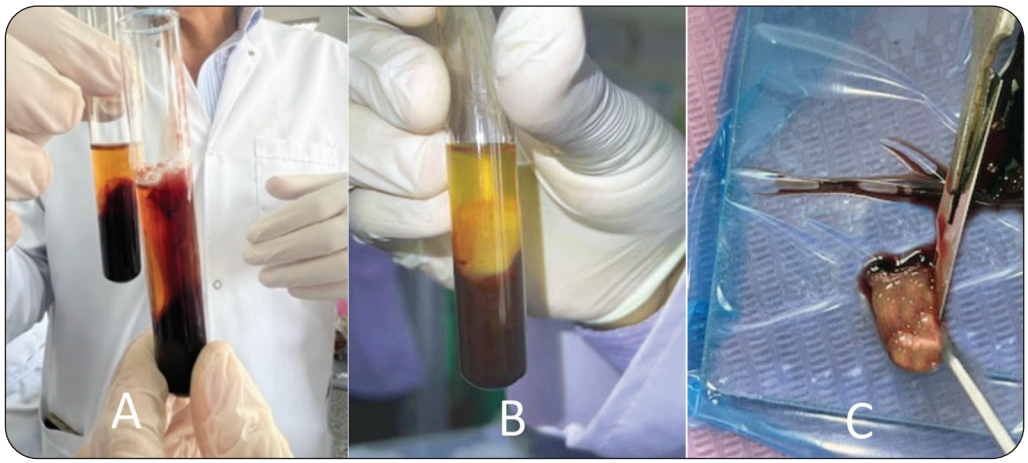

Fig. (1) Picture showing the preparation of PRF (a) Blood after centrifugation at 3000 rpm, (b) PRF after separation, (c) Cutting PRF

The tooth was recessed. PRF was introduced into the canal and carried to the apical part of the root canal using an endodontic plugger. The access cavity was then sealed with the glass ionomer cement followed by a glass ionomer filling (EQUIA FORTE bulk fill glass hybrid restoration CG JAPAN) (Fig. 2).

Fig. (2) Picture showing the Introduction of PRF inside the root canal

Group II used both blood clot and MTA (mineral trioxide aggregate).

A sterile 21-gauge needle was used to induce bleeding in the canal. A cotton pellet was then inserted to wait for clot formation. MTA (manufactured by Angelus in Londrina, Brazil) was used to seal the canal and a glass ionomer filling was used to seal the cavity.

A periapical X-ray was taken as a base. The tooth was evaluated using X-rays at 3, 6, and 12 months after the procedure.

The radiographic outcome was evaluated using intraoral digital periapical radiographs. The Ørstavik's Periapical Index [16] (Table 1) was used to assess the periapical radiolucency, while the Chen and Chen Index [17] (Table 2) was used to assess the apical response at 3, 6, 9, and 12 months during the follow-up period.

Table (1) *Displays Ørstavik's Periapical Index used for diagnosing periapical radiolucency*

Score	Criteria
1	Normal periapical structures
2	Small changes in bone structure
3	Changes in bone structure with some mineral loss
4	Periodontitis with well-defined radiolucent areas
5	Severe periodontitis with exacerbating features

Table (2) *Chen and Chen Index for apical response*

Score	Criteria
Type 1	Increased thickening of the canal walls and continued root maturation.
Type 2	No significant continuation of root development with root apex becoming blunt and closed.
Type 3	Continued root development with the apical foramen remaining open.
Type 4	Severe calcification (obliteration) of the canal space.
Type 5	A hard tissue barrier formed in the canal between the coronal MTA plug and the root apex.

Reliability of the research

By utilizing intra-class correlation coefficients (ICC) with a two-way mixed and absolute agreement model, both intra-examiner and inter-examiner reliabilities were fully assessed. This approach enables us to confidently evaluate the accuracy and consistency of the results, ultimately ensuring that you receive the highest quality data possible.

The data were analyzed and the Chi-square (X2) test was used to compare proportions between two qualitative parameters.

RESULTS

At the beginning of our research, we selected 20 patients at different periods. However, four parents refused to sign the consent form, and two children showed poor oral hygiene one month after the procedures, with severe gingival inflammation plaque induced. Additionally, two patients failed to come for the follow-up, so we excluded six patients. Fortunately, we were able to replace four of them, and finally, we ended up with 14 patients divided into two groups, each consisting of seven patients.

The age of the selected children was from 9-12 with a mean of 10 years for group I and 9.86 for group II as shown in Table (3).

Table (3) *Age group of all participant children in both groups*

Age	Group I	Group II
Range	9 – 12	9 – 12
Mean ± SD	10.0 ± 1.15	9.86 ± 1.21
T. test	0.225	
P. value	0.825	

Five males (71.4%) and two females (28.6%) children selected for each group as shown in Table 4.

Table (4) *Sex distribution in the groups*

Sex	Group I	Group II
Male (%)	5 (71.4%)	5 (71.4%)
Female (%)	2 (28.6%)	2 (28.6%)
X^2. test	0.0	
P. value	1.0	

The apical closure results were evaluated according to Ørstavik's Periapical Index, and the following table shows the apical response. Table (5)

Regarding the apical closure, all the selected cases had a score of 2 which showed small changes in the periapical structures After three months the score remained as it was for both groups, but after six months there was a big difference noticed between the two groups as 6 cases from group I (85.7%) showed improvement of the score of periapical structure with a score 1 while only one case (14.5%) remains on score 2.

Group II periapical changes after six months showed 4 cases (57.1%) of changes to normal periapical structures while three cases still showed some periapical changes (score 2).

Twelve months follow up the periapical changes score remained the same for group I while in group II two cases (28.6%) changed to score 3 with changes in bone structure with some mineral loss. As shown in Table 5 and Table 6.

Table (5) *Results of periapical changes according to Ørstavik's Periapical Index*

Ørstavik's Periapical Index	Group I	Group II	X^2	P. value
Normal periapical structures (%) 1	5 (71.4%)	5 (71.4%)		
Small changes in bone structure (%) 2	2 (28.6%)	2 (28.6%)		
Changes in bone structure with some mineral loss (%) 3				
Periodontitis with well-defined radiolucent areas (%) 4				
Severe periodontitis with exacerbating features (%) 5				

Table (6) *Follow-up results for periapical changes according to Ørstavik's Periapical Index*

Ørstavik's Periapical Index		Group I	Group II	X^2	P. value
Baseline	Small changes in bone structure	7 (100%)	7 (100%)	-	-
3 months	Small changes in bone structure	7 (100%)	7 (100%)	-	-
6 months	Normal periapical structures	6 (85.7%)	4 (57.1%)	1.400	0.237
	Small changes in bone structure	1 (14.3%)	3 (42.9%)		
12 months	Normal periapical structures	6 (85.7%)	4 (57.1%)	2.400	0.301
	Small changes in bone structure	1 (14.3%)	1 (14.3%)		
	Changes in bone structure with some mineral loss	0 (0%)	2 (28.6%)		

Regarding apical response, it was evaluated according to the Chen and Chen Index, and after three months the evaluation showed no periapical changes for both groups but after six months three cases of group one (42.9%) showed increased thickening of the root canal walls and continued root maturation (type I) while four cases (57.1%) showed continued root development with apical foramen remains opened (type III).

Six months group II follow-up showed three cases (42.9%) with root development continued while the apical foramen still opened, while two cases (28.6%) with no significant continuation of root development with root apex becoming blunt and closed (type 2), two cases (28.6%) with continued root maturation and increased thickening of the canal walls (type 1).

After one year, all cases of group I (100%) showed thickening of the canal walls and root formation completed, while in group II only three cases showed increased thickening of the walls with roots still in maturation, two cases (28.6%) with no significant continuation of root development with root apex blunt (type 2) while the other two cases (28.6%) showed the formation of hard tissue barrier under MTA (type5) as shown in table (VII)

Radiographic and clinical photos for the results of the research are shown in Figures 3, 4, 5, and 6.

Table (7) *Comparison between two groups according to Chen and Chen index*

Chen and Chen Index for apical response		Group I	Group II	X^2	P. value
3 months	No changes	7 (100%)	7 (100%)	-	-
6 months	Type 1	3 (42.9%)	2 (28.6%)	2.343	0.310
	Type 2	0 (0%)	2 (28.6%)		
	Type 3	4 (57.1%)	3 (42.9%)		
12 months	Type 1	7 (100%)	3 (42.9%)	5.600	0.061
	Type 2	0 (0%)	2 (28.6%)		
	Type 5	0 (0%)	2 (28.6%)		

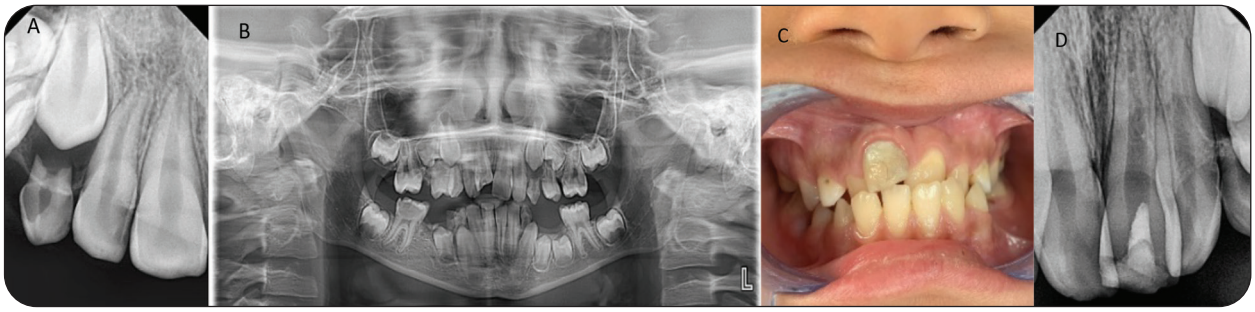

Fig. (3) Photos for a preoperative case from group I (a) Periapical Xray (b) Panoramic X-ray (c) clinical photo of the injured tooth (d) X-ray immediately postoperative

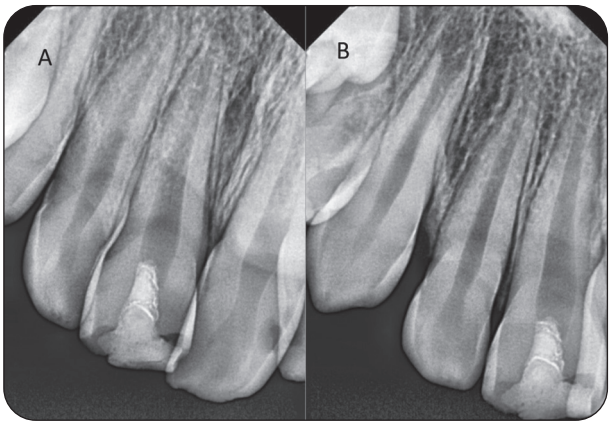

Fig. (4) Photos of periapical x-rays taken three (a) and six (b) months postoperative show thickening of root canal walls

Fig. (5) Postoperative photos for a patient from group I showed complete apical closure

Fig. (6) Photo of an x-ray for a case from group II after 12 months showed the calcific barrier under the MTA layer

DISCUSSION

Stem cell technology has revolutionized the medical field, and the dental field is following suit with the development of many techniques based on stem cell technology.

Stem cell technology has brought about significant advancements in the treatment of non-vital immature permanent teeth. The old techniques relied on non-vital pulp therapy which had several drawbacks. However, with the new technology,

complete root formation is now possible, avoiding the disadvantages of the old techniques. [18]

The treatment of nonvital immature teeth in children is an extremely difficult process for pediatric dentists. When permanent teeth grow, they only achieve 60% to 80% of root development. Unfortunately, this age period is characterized by high levels of children's activity, which makes them more susceptible to injuries. Children with poor oral hygiene may develop caries that can quickly lead to incomplete root development after teeth eruption due to infection and loss of pulp vitality. [19]

The most common treatment options, such as calcium hydroxide and MTA apexification, do not always deliver the ideal treatment result that is expected for certain teeth. While these options help with apical closure, they fall short of continuous root formation and mineralized structures within the root canal. For these cases, the regenerative approach is considered the treatment of choice. It results in the continuous formation of the root and mineralized structures within the root canal, making it the ideal option. [20]

In this study, we used two different scaffolds for pulp regeneration of young permanent teeth: PRF in group I and a blood clot in group II.

Hertwig's epithelial root sheath is considered to be a significant source of stem cells in the periapical area of the root. In the revascularization method, bleeding is intentionally induced from the periapical area to serve as a scaffold for pulp regeneration, supporting our design for the second group of patients. Some theoretical research suggests that this method could be effective in the presence of apical periodontitis or periapical infection. It relies on the apical papilla as a source of stem cells, which offers the main advantage of not being closely attached to the dental pulp. This gives it the capacity for regeneration due to its rich stem cells and the continuation of root development. [8,21,22,23]

In contrary to other researchers who think that this procedure is traumatic and may cause irritation and discomfort for children and at the same time difficulty in bleeding control which is proved by Petrino et al. [24] and Ding et al. [25] case series [26]

The disadvantages of the traditional revascularization method have led researchers to consider a more biologically acceptable approach which we considered in our study as we used platelet-rich fibrin (PRF) as a scaffold in the first group. [24]

Regarding the results of our study, we choose the age group from 9-12 to be sure of the eruption of the anterior teeth and the formation of roots.

The periapical area changes showed that there was an improvement in the periapical areas for both groups up to 85.7% improvement to normal structure after 12 months for group I patients, while in the second group, four cases showed normal improvement and one with little periapical tissue changes while two with more radiolucent changes with no significant statistical difference between the two groups. This is coordinated with the results of a study by Sharma *et al* [27]2016 who conducted a study on 16 necrotic immature permanent maxillary incisors which were selected for regenerative endodontics. The results of their study demonstrated that there was an improvement in terms of periapical healing, apical closure, root lengthening, and dentinal wall thickening. PRF gave better results than blood clots in terms of periapical healing, apical closure, and dentinal wall thickening. On the opposite side, Sudha and Nageswara 2020 [28] in their study found that in group I, (PRF) 90% of teeth had fair and 10% had good root lengthening, in group II, (blood clot)20% had fair and 80% had good root lengthening and in group III, 71% had fair and 29% had good root lengthening. None of the group had excellent root lengthening. Nosratet al [29] in their study demonstrated that teeth with the presence of blood clot in their canals had better radiographic treatment outcome as compared to

teeth without it. Apical closure as well as thickening of dentinal walls were better in these cases, which is also not coordinated with our results which showed better results with PRF.

Regarding The root canal wall thickness and apical part closure which were evaluated using the Chen and Chen index the results showed that all the teeth in group I with closed apex and thickening of the root canal walls at the end of the research (12 months), while in group II only three cases (42.9%) with complete apical closure while two cases with continuous root formation but without any failure and finally two cases with hard tissue formation between the MTA layer and root apex, However, several studies report that root maturation is unpredictable, and depends upon the trauma and severity of the periapical lesion both of which may disturb the biological function of Hertwigs epithelial root sheath and its interaction with mesenchymal stem cells in the dental follicle. [30]

In a study done by. Murray et al 2007 for regeneration in three cases, the thickening of the dentin and canal walls on regeneration with PRF during the follow-up period is consistent with the observations in the relevant literature. [31]

Coincide with these results Çelik et al., 2022[32] In the regenerative endodontic treatment with PRF, the infections in the periapical region had healed, the root walls had thickened, and found that the root tips had closed because of continued root development. These results are consistent with the relevant literature.

Wakhloo et al 2022 [12] results observed the changes in the open apex in the postoperative radiographs coincided with type 5 of Chen's response, which is the formation of a hard tissue barrier at various levels in the root canal between the coronal biodentine and the root apex. The hard tissue barrier was visible radiographically after 3 months, in our study this happened with two cases from the second group with the formation of hard tissue related to the MTA layer but after 12 months follow-up in contrast to the study conducted by Santhakumar M,2018[33] which reports no radiographic change at 6 months follow-up examination. and in contrast with our first group study.

The overall success rate in this study was similar to that of Sedwick [34], but higher than those of Saoud et al., [35] Jeeruphan et al., [36] and Cehreli et al. [37]. These findings are consistent with many other clinical and radiographic success outcomes from various other studies. [35, 38, 39] On the other hand, many other research studies have reported a high failure rate, including both clinical and radiographic failures. [40]

These different studies varied in terms of the type of study conducted and the factors considered to evaluate the success or failure of the procedures. [35, 37, 38, 40, 41]

The conflict regarding these procedures revolves around successful procedures that are highly recommended, and on the other side, failed procedures that are not recommended, possibly due to various reasons reported by Almutairi et al. [40] and Lee et al. [42]:

1. The absence of a commonly followed technique
2. Inadequate and non-standardized disinfection protocols
3. Lack of clarity and proper definition in the healing process
4. Variations in the scaffolds used

We think that PRF is a vital source of stem cells and growth factors that will help in the regeneration of the pulp, but the problem usually lies in steps, especially regarding blood sample collection, which is fearful with most children and not comfortable with their parents.

CONCLUSION

The revascularization method of treating immature root canals has superior properties compared to other techniques. It allows for continued root formation and creates a favorable environment for the future deposition of dentin on the canal walls. Additionally, it is a more effective and simple technique. [22]

"Both blood clot and PRF are good for pulp regeneration, but growth factors are one of the most important elements in the revascularization process. When the used material is rich in these factors or has a higher capacity to induce their secretion, there will be a higher success rate. That is why we consider PRF to be better for regeneration than blood clots, and this may need more research.

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تقييم الفيبرين الغني بالصفائح الدموية (PRF) كسقالة في تجديد اللب في الأسنان الأمامية الدائمة الشابة

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الملخص :

الهدف: تم تصميم هذه الدراسة لتقييم الفيبرين الغني بالصفائح الدموية (PRF) باعتباره سقالة في تجديد اللب في الأسنان الأمامية الدائمة الشابة.

المواد والاساليب: تم إجراء عملية إعادة التوعي في 16 حالة من الأسنان الدائمة غير الناضجة في جميع الحالات الـ 16، وتم إجراء التطهير داخل القناة بأجهزة ميكانيكية محدودة، والري الوفير، وهيدروكسيد الكالسيوم، في الموعد النهائي، تم إحداث نزيف داخل القناة. في إحدى المجموعات، سيتم الحصول على PRF عن طريق الطرد المركزي للدم المسحوب من المرضى، ويتم وضعه بشكل جيد باستخدام سدادة في قناة الجذر حتى يصل إلى المستوى الإكليلي 3/1 من القناة، وفي المجموعة الثانية سيتم تهتك المنطقة المحيطة بالذروة، يتم إجراء ذلك لتحفيز النزيف ثم يتم استخدام MTA للتلامس المباشر مع الأنسجة ثم يتم ترميم التجويف باستخدام الأسمنت المتماثرات الشاردة الزجاجية. وسيتم إجراء المتابعة السريرية والشعاعية على فترات 3،6،9 و 12 شهرًا.

النتائج: عد المتابعة لوحظ أن المجموعة الأولى (PRF) حصلت على نتائج شعاعية أفضل مقارنة بالمجموعة الثانية (جلطات الدم). أظهرت المجموعة الأولى أيضًا شفاءًا ممتازًا حول الذروة، واكتمال الجذور، وسماكة جدران قناة الجذر. ومع ذلك، شهدت المجموعة الثانية تأخيرات في عملية التجديد.

الخلاصة: قد يكون العلاج اللبي التجديدي بديلاً جيدًا لعلاج الأسنان الدائمة الفتية بدلاً من الطرق التقليدية التي تؤدي إلى قمة مغلقة دون أي نمو للجذر.

الكلمات المفتاحية: شاب دائم، الفيبرين الغني بالصفائح الدموية، إعادة الأوعية الدموية، التجدد اللبية