

REVASCULARIZATION INDUCED MATUROGENESIS OF NON-VITAL IMMATURE TEETH USING DIFFERENT SCAFFOLDS AND INTRA CANAL MEDICATIONS

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ABSTRACT

Purpose: to clinically and radiographically evaluate revascularization induced maturogenesis of non-vital immature teeth using Platelets rich plasma (PRP) compared to blood clot as scaffolds with two different intracanal medications [modified triple antibiotic paste (mTAP) and calcium hydroxide (Ca(OH)₂) mixed with chlorhexidine (CHX)]. **Materials and Methods:** 32 patients aged between 9 and 20 years requiring endodontic therapy of maxillary central incisors were selected for this study. Patients were randomly divided into four equal groups (n=8) according to type of scaffold and intracanal medication used in the treatment as; Group I: Blood clot scaffold/ mTAP intra canal medication ,Group II: PRP scaffold/mTAP intra canal medication, Group III: Blood clot scaffold/Ca(OH)₂ mixed with CHX intra canal medication, Group IV: PRP scaffold/ Ca(OH)₂ mixed with CHX intra canal medication. Patients were evaluated clinically for presence or absence of spontaneous pain, pain on palpation, swelling, sinus tract or crown discoloration and radiographically for any change in apical foramen diameter, root length and root canal diameter at a predetermined point at 15mm from the incisal edge. Radiographic digital examination was done immediately postoperative and at 3,6,9 and 12months of follow-up under the same circumstances. **Results:** Clinically, all patients were completely asymptomatic through out the study. Radiographically, concerning percentage of root length increase, PRP produced superior results with statistically significant difference compared to blood clot as a scaffold while mTAP showed significantly better results compared to Ca(OH)₂ mixed with CHX as intracanal medication. Concerning percentage of canal diameter decrease and apical foramen diameter decrease, using PRP scaffold with mTAP medication produced superior results with statistically significant difference when compared to blood clot scaffold with a mix of Ca(OH)₂ and CHX medication respectively. **Conclusions:** PRP is better than Blood clot as a scaffold and mTAP is better than Ca(OH)₂ mixed with CHX as an intracanal medication when they are used during revascularization of immature teeth.

KEYWORDS: Calcium hydroxide mixed with chlorhexidine, Immature teeth, Modified triple antibiotic paste, Platelets rich plasma, Revascularization.

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INTRODUCTION

Loss of dental pulp tissue vitality has serious consequences, particularly in young patients, where pulp necrosis results in arrested root development. Large open apices and thin funnel shaped root canal walls are responsible for complications such as insufficient root canal debridement, improper obturation or root fracture in immature teeth.

For decades, calcium hydroxide ($\text{Ca}(\text{OH})_2$) apexification has been used as an effective technique to treat immature teeth. It is the least expensive technique to induce a calcified apical barrier. However, this long term procedure does not increase the thickness or length of the root and causes the tooth to be susceptible to future fractures^[1].

Mineral trioxide aggregates (MTA) apexification is considered as an effective alternative technique for $\text{Ca}(\text{OH})_2$ apexification. The most important advantage of this technique is to reduce the number of sessions required to create an apical barrier. However, this technique has the same disadvantages as $\text{Ca}(\text{OH})_2$ of incomplete root development and fragile root structure^[2].

Another concept called pulp regeneration has been introduced to treat necrotic immature permanent teeth. It has different applied regenerative endodontic techniques as: Root canal revascularization, Stem cell therapy or Gene therapy. However, revascularization is considered the most easily applied regenerative technique^[3].

The success of revascularization technique depends on three key factors: root canal disinfection, quality of the used scaffold, and hermetic coronal seal. Eradication of infection from the root canal space is the first step to obtain successful revascularization. One of the most widely used intracanal medicaments is triple antibiotic paste (TAP) which is one of the combinations that are effective against the most common bacterial species in infected root canals^[4]. TAP contains both bactericidal (metronidazole, ciprofloxacin) and bacteriostatic

(minocycline) agents. However, the presence of minocycline has been associated with tooth discoloration, so minocycline may be replaced with amoxicillin or cefaclor forming modified triple antibiotic paste (mTAP)^[5].

2% chlorhexidine gel (CHX) is an active vehicle that provides additional antimicrobial properties to $\text{Ca}(\text{OH})_2$ so this mix was introduced as an alternative intracanal dressing to improve the antimicrobial activity specially against *E. faecalis* and *C. albicans* which are resistant to $\text{Ca}(\text{OH})_2$ alone^[4].

Proper scaffolds are the second key factor for success of revascularization. They are three dimensional porous solid structures which provide support to the regenerated tissue. Traditionally, blood clot was used as a scaffold utilizing the fibrin matrix within it to trap cells necessary for tissue regeneration. In addition, its rich content of growth factors, autologous nature, safety profile, and lower cost cause the blood clot to be considered as a successful scaffold^[6].

On the other hand, Platelet-rich plasma (PRP) is an autologous platelet concentrate with a rich source of growth factors. It has several advantages of easy preparation, high platelet concentration, and rich fibrin matrix which allows growth factors entrapment^[7].

Clinical and radiographic evaluation can be used for assessment of induced maturogenesis associated with revascularization. Clinical evaluation assesses presence/absence of spontaneous pain, pain on palpation, swelling, sinus tract or crown discoloration. While radiographic evaluation assesses any changes in dentin thickness, root length, apical foramen size and periapical lesion size^[8]. Radiographic evaluation can be applied using different methods as intraoral periapical digital paralleling technique or cone beam computed tomography. However, Intraoral paralleling technique provides a less complicated, less hazardous, and more available method for evaluation with reduced cost during follow up periods^[9].

There are limited literatures comparing the combined effect of different scaffolds and intracanal medications during treatment of incompletely formed roots. So, this study aimed to evaluate clinically and radiographically revascularization induced maturogenesis of non-vital immature teeth using PRP compared to blood clot as scaffolds and mTAP or $\text{Ca}(\text{OH})_2$ mixed with 2% CHX as different intracanal medications.

MATERIALS AND METHODS

The minimum number of sample size for this study was 28 patients. The sample was collected based on a previous study conducted by Turkey et al.,⁹ The significance level was 0.05 and the power sample size was more than 80% for this study and the confidence interval 95% and the actual power is 96.7%. The sample size calculated using a computer program G power version 3.

The formula of sample size
$$\text{sample size} = Z^2 P(1-P) / C^2$$

Where:

Z = Z value (1.96 for 95% confidence level)

p = percentage picking a choice, expressed as decimal

c = confidence interval, expressed as decimal.

An over sizing of the sample was done to increase the validity of the results, so the sample size was 32.

32 patients aged between 9 and 20 years requiring endodontic therapy of maxillary central incisors were selected for this study. The purpose of the present study was explained to the patients or their legal guardians and informed consents were obtained according to the guidelines on human research adopted by the Research Ethics Committee at Faculty of Dentistry, Tanta University. Thirty-two consenting patients were allocated randomly into one of the four treatment groups. An independent, trained investigator not involved in the study handled the randomization and concealment process.

Random sequence generation was achieved using a computer random allocation program and concealed from the operator using the sequentially numbered opaque sealed envelope (SNOSE) technique. Then a closed envelope containing the instruction to use either mTAP medication/blood clot scaffold, mix of $\text{Ca}(\text{OH})_2$ and CHX medication/blood clot scaffold, mTAP medication /PRP scaffold or mix of $\text{Ca}(\text{OH})_2$ and CHX medication/PRP scaffold was selected. The patients were not aware of the treatment medication or scaffold they received. Hence, this was a single-blinded trial. The inclusion criteria were restorable immature non-vital maxillary central incisors either due to trauma or caries, teeth with/without signs and/or symptoms of periapical pathology, medically free patients and preoperative radiograph showing incomplete root formation with apical foramen minimally at stage 8 according to Nolla's scoring system for apical foramina development [10]. While the exclusion criteria included fractured root, non-restorable teeth, grade III mobility, abnormal anatomy, external or internal root resorption, retreatment cases, immunocompromised patients and patients with allergic response to any medication or materials used in the study.

Patients were randomly divided into four equal groups (n=8) according to the type of the used scaffold and intracanal medication used in the treatment: Group I: Blood clot scaffold / mTAP intra canal medication, Group II: PRP scaffold / mTAP intra canal medication, Group III: Blood clot scaffold / $\text{Ca}(\text{OH})_2$ mixed with CHX intra canal medication, Group IV: PRP scaffold / $\text{Ca}(\text{OH})_2$ mixed with CHX intra canal medication.

Preoperative intraoral periapical digital radiograph using paralleling technique was made for each patient using digital intraoral sensor*. Teeth were anaesthetized using plain local anaesthesia**. After rubber dam isolation, teeth were cleaned and

* Dr.Suni plus Digital Intraoral Sensor, Suni Medical Imaging, Inc., Sanjose, USA.

** Scandonest 3% plain; septodont Saint-Maur-Des-Fosses, France

disinfected with, 10% povidone-iodine betadine antiseptic solution*, then the straight-line access was performed. Working length was determined then root canals were irrigated using 20 mL of 1.5% NaOCl solution** followed by 20 mL of 17% EDTA*** solution with intermediate rinse of 10 mL sterile saline using 30-gauge side-vented needle**** adjusted at 3mm shorter than the working length [9].

For groups I and II, mTAP intracanal medication was prepared using metronidazole***** (500 mg) tablets, ciprofloxacin***** (500 mg) tablets and cefaclor***** (500 mg) capsules in a ratio of 1:1:1 [11], then the powder was mixed with saline drops in 1:3 ratio [146]. For groups III and IV, a mixture of $\text{Ca}(\text{OH})_2$ ***** and CHX***** was used as an intracanal medication. A creamy dressing was prepared from $\text{Ca}(\text{OH})_2$ paste and 2% CHX gel in a proportion of 1:1.

For all groups, the canal space was dried using paper points*****, then the prepared paste was injected into the canals using a sterile plastic syringe with a 20-gauge open-ended needle***** adjusted to fill the canal space except the most apical 3mm^[9]. The access cavities were double sealed by a 0.5-1mm thick layer of chemically cured glass ionomer***** filling followed by light cure composite resin filling

material. Then it was radiographed to check the position and sealing of the restoration and left for 3 weeks^[5].

On the next visit after 3 weeks, after anaesthesia, isolation and tooth disinfection, the restoration was removed and the canal was irrigated with 10mL of 1.5% sterile NaOCl solution followed by 10mL of 17% EDTA solution with intermediate rinse of 10 mL of sterile saline solution using 30-gauge side-vented needle adjusted at 3mm shorter than the working length alternatively with minimal instrumentation using manual k-file # 20*****¹⁸ fitted at 3mm shorter than the working length. A final flush of 10 mL of sterile saline solution was made and canals were dried with sterile paper points to the full working length to be ready to receive scaffolds.

For groups I and III, using blood clot scaffold, over-instrumentation was done using manual stainless-steel k-file #40 adjusted to be extended 2mm beyond the apex to induce bleeding and it was left for five minutes to ensure blood clot formation. Then excess blood clot was wiped using sterile paper point #80 to clean the most coronal 3mm of the root canal to ensure that blood clot is located nearly at 3mm below the orifice (Fig.1).

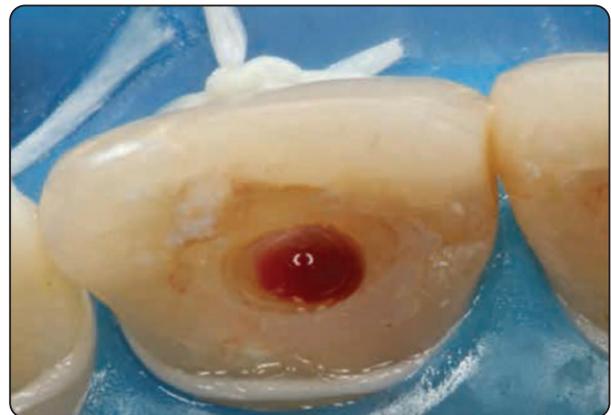


Fig. (1) Induced bleeding within the root canal

*The Nile Co. for Pharmaceuticals and Chemical Industries, A.R.E

** Prime Dental products, kalher, India

*** PrevestDenPro Limited, Digiana, India

**** Transcoject, Neumünster, Germany

***** Flagyl 500mg, Aventis, Cairo, Egypt

***** Cipromax 500mg, El-Dawaia company, alkosim, Saudi Arabia

***** Cefaclor capsules; Pharco B International, Alexandria, Egypt

***** Metabiomed Co LTD, Osongsaengmyeong, Korea

***** Endogel, Itapetininga, Sao Paulo, Brazil

***** DiaDent, Chongju City, Korea

***** Moscomid, Tanta, Egypt

***** Medifil, Promedica, Neumünster, Germany

*****¹⁸Mani, Inc, Tochigi, Japan

For groups II and IV, PRP was early prepared by drawing 8 ml of blood of the patients and collecting it in a 10mL sterile glass tube containing acid citrate dextrose as an anticoagulant. Then, it was centrifuged using laboratory PRP tube box dental centrifuge machine^{*19} at 2400 rpm for 10 minutes to separate PRP and platelet-poor plasma (PPP) from the red blood cell fraction.

By the end of the first centrifugation cycle, the tube contained 3 layers; a bottom red thick layer containing red blood cells, a central buffy lightly yellowish layer containing white blood cells and plasma cells, and a supernatant turbid liquid layer which is acellular plasma (Fig.2). Supernatant layer (PRP+PPP) was transferred to another tube and centrifuged again at 3600rpm for 15 minutes. At the end of this cycle, PRP precipitated at the bottom of the tube as light yellowish slimy viscous substance which was retrieved from the tube using plastic instrument. It was placed on a sterile glass petridish, then mixed with 1 ml of 10% calcium chloride (Fig.3)¹⁷.

PRP scaffold was introduced into the pulp chamber using sterile cotton pliers and carried to the whole root canal to the full working length except the most coronal 3mm using a # 40 finger plugger to create a space for MTA^{**20} placement.

For all groups, MTA was mixed in a powder / water ratio 3:1, which was used as orifice plug to seal the orifice and the coronal 3mm of each root canal. Immediate digital periapical radiograph was taken to ensure position and extent of MTA. It was covered by moist cotton pellet and temporary restoration for 24 hours, then the patient was recalled for replacement of the temporary restoration^{***21} by composite resin.

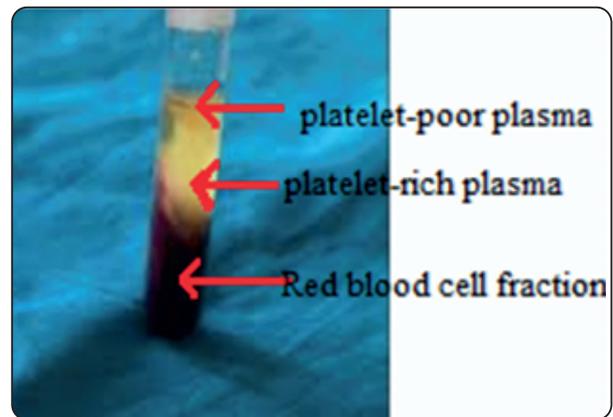


Fig. (2) Layers of platelet-rich plasma, platelet-poor plasma and red blood cell fraction after centrifugation

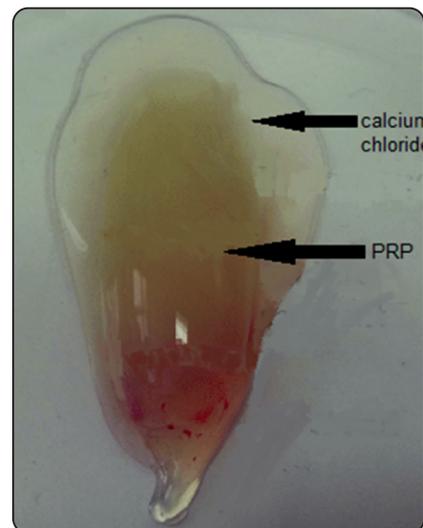


Fig. (3) Calcium chloride added to PRP

After final restoration, immediate postoperative periapical digital radiograph was taken as baseline data. The patients were recalled at 3, 6, 9, 12 months for clinical and radiographic evaluation. Clinical assessment criteria include presence or absence of spontaneous pain, pain on palpation, swelling, sinus tract or crown discoloration. Follow up radiographs (Fig.4) were taken with the same angulation as the immediate postoperative one to evaluate any change in apical foramen diameter, root length, root canal diameter at a predetermined point at 15mm (57 pixels) from the incisal edge

* ¹⁹Laboratory PRF. PRP tube box dental centrifuge, Manson Inc., Beijing, China

** ²⁰MTA, Angelus, Londrina, Brazil

***²¹MD-Temp, Metabiomed Co LTD, Osongsaengmyeong, Korea

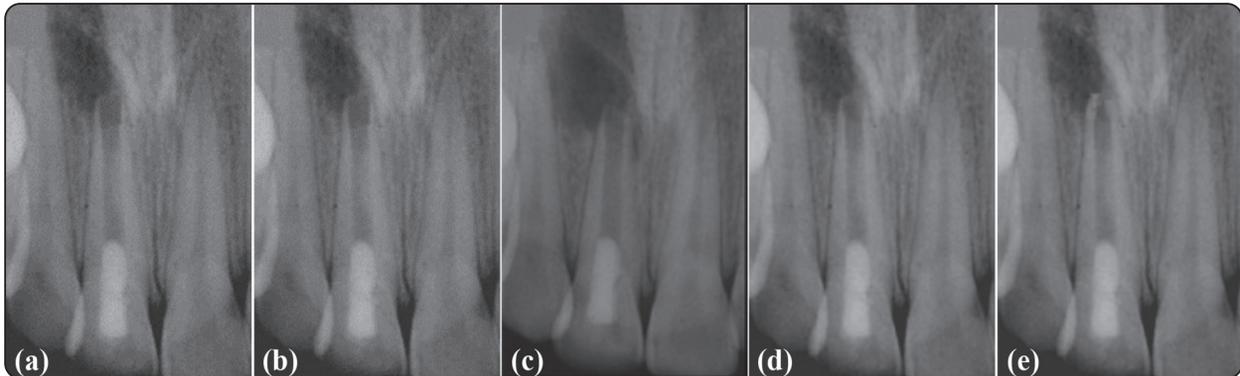


Fig. (4) Immediate postoperative and follow up radiographs at 3,6,9,12 months respectively (a,b,c,d and e)

using Image J software^{*22}. Root length and diameter were measured in pixels which were transformed later to millimeters according to the equation (1pixel=0.264583mm).

Regarding root length, canal width and apical foramen diameter any changes in measurements between the immediate postoperative and frequent follow up scores were calculated using percent of change formula^[12]: $[\Delta \text{ or change in value} / \text{base line value}] \times 100$.

Statistical analysis was performed using One-Way Analysis of Variance (One-Way ANOVA) with SPSS software (version 21)^{**23} to determine significance differences among groups, then multiple pairwise comparisons were performed using Tukey's test to compare the data with significance level of $p < 0.05$.

RESULTS

Clinical and radiographic evaluations after follow-up for 12 months showed that all cases had improved as compared to the baseline levels.

Clinical evaluation

Clinically, the four groups showed satisfying results; all patients were completely asymptomatic

through out the study period with no tenderness to palpation or percussion. No preoperative or postoperative swelling was presented in any of the included patients and there was no crown discoloration detected.

Radiographic evaluation

Radiographically, all teeth in all groups showed improvement in terms of root length increase, canal diameter decrease, and apical foramen diameter decrease.

Results of root length increase:

Table 1 showed comparison of percentage of root length increase among the four tested groups at each time interval revealed that group II showed the highest percentage of root length increase followed by group IV, group I and group III at all time intervals (6,9,12 months) except at 3 months. At this time interval, groups II and IV showed similar percentage of root length increase while groups I and III didn't show any increase in root length. Statistical analysis using One-Way ANOVA revealed a non statistically significant difference among the tested groups at 3 and 6 time intervals. Concerning 9 and 12 months time intervals, Tukey's pair-wise test was performed and revealed that there were statistical significant differences among group I versus II, group II versus III, and group II versus IV (Table 2).

* ²²Image J, 50cNIH.USA

** ²³SPSS Inc. Chicago, USA.

In addition, comparison of percentage of root length increase among different tested follow up time intervals (3, 6, 9, 12 months and immediate postoperative) for each tested group was performed (Table 1) regarding all groups, the percentage of root length increase values were arranged from the highest to the lowest at 12, 9, 6, 3 months and immediate postoperative follow up time intervals respectively.

One-Way ANOVA revealed a statistical significant difference among the four follow up time intervals in all groups regarding the percentage of root length increase ($P \leq 0.001$). Tukey’s pair-wise test revealed that there were statistical significant

differences among all the follow up time intervals with P -value <0.001 as shown in Table 3. This means that at all tested groups, there were statistically significant increase in root length throughout the follow up periods

Furthermore, comparison between tested medications regardless of the type of scaffolds and time intervals, revealed that mTAP medication showed better results as it recorded higher percentage of root length increase compared to the other medication. T-test was performed and revealed no statistically significant difference between both tested medications ($P>0.05$) (Fig.5).

TABLE (1) Percentage of root length increase and its mean \pm SD of the four tested groups at different follow up time intervals (3, 6, 9, 12months and immediate postoperative) and their statistical analysis

Root length Time interval	Group I Blood clot / mTAP		Group II PRP/ mTAP		Group III Blood clot / Ca(OH) ₂ +CHX		Group IV PRP + Ca(OH) ₂ +CHX		ANOVA	
	Mean \pm SD	% Of change	Mean \pm SD	% Of change	Mean \pm SD	% Of change	Mean \pm SD	% Of change	P-value	
									Mean \pm SD	% Of change
Immediate postoperative	18.087 \pm 0.502		18.703 \pm 0.722		17.709 \pm 0.937		18.074 \pm 0.780		0.126	
Post 3 Months (p-3)	18.087 \pm 0.502	0.00	18.704 \pm 0.722	0.01	17.709 \pm 0.844	0.00	18.075 \pm 0.780	0.01	0.128	0.580
Post 6 Months (p-6)	18.126 \pm 0.507	0.21	18.748 \pm 0.719	0.24	17.745 \pm 0.937	0.20	18.116 \pm 0.752	0.23	0.123	0.445
Post 9 Months (p-9)	18.150 \pm 0.504	0.34	18.830 \pm 0.716	0.67	17.765 \pm 0.840	0.31	18.153 \pm 0.754	0.44	0.255	<0.001*
Post 12 Months (p-12)	18.219 \pm 0.505	0.72	18.902 \pm 0.715	1.06	17.833 \pm 0.930	0.70	18.215 \pm 0.754	0.78	0.151	<0.001*
ANOVA P-value	0.981	<0.001*	0.996	<0.001*	0.902	<0.001*	0.992	<0.001*		

TABLE (2) Pair-wise statistical analysis of percentage of root length increase of tested groups at 9 and 12 months follow up time intervals

		TUKEY’S Test					
		I&II	I&III	I&IV	II&III	II&IV	III&IV
% of	P-9M	<0.001*	0.153	0.145	<0.001*	0.027*	0.144
Root length increase	P-12M	<0.001*	0.156	0.153	0.030*	0.034*	0.151

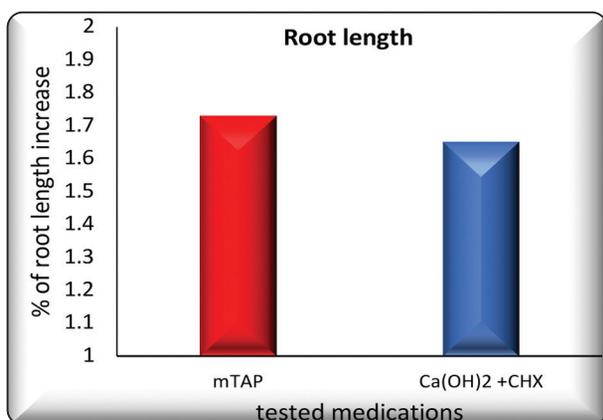


Fig. (5) Bar chart showing percentage of root length increase of mTAP medication versus Ca(OH)₂ mixed with 2%CHX regardless the type of scaffolds and time intervals

When the both tested scaffolds were compared regardless of the type of medication and time intervals, better results were obtained whenever revascularization was stimulated using PRP scaffold compared to using blood clot scaffold where they recorded percentage of root length increase with a statistical significant difference between them ($P=0.001$) (Fig.6).

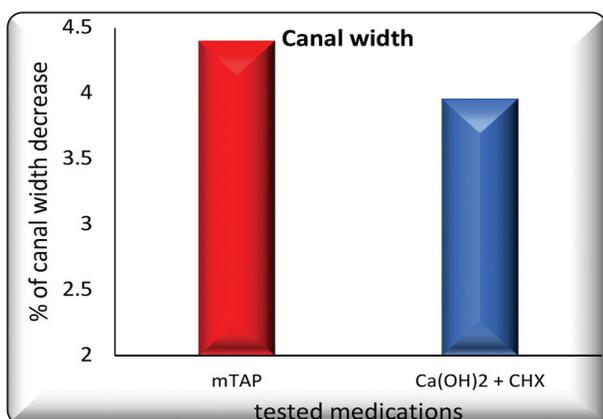


Fig. (6) Bar chart showing percentage of root length increase of PRP scaffold versus blood clot scaffold regardless the type of medications and time intervals

Results of root canal width decrease:

Comparison of percentage of root canal width decrease among the four tested groups at each time interval revealed that group II showed the highest percentage of canal width decrease followed by group IV, group I and group III at all time intervals

except at 3 months follow up period where both groups I and III didn't show canal width decrease while groups II and IV showed 0.02% of canal width decrease as shown in Table 4. One-Way ANOVA test didn't reveal any statistically significant differences among the tested groups at 3 months. There were statistically significant differences among tested groups at 6, 9 and 12 months. So, Tukey's Pair-wise comparison test was performed among the tested groups as shown in Table 5.

Percentage of canal width decrease values of the four follow up intervals (3, 6, 9, 12 months in comparison to immediate postoperative) were compared at each group as presented in Table 4, regarding groups I, III, the highest percentage of canal width decrease was recorded at 12 month time interval while no change was recorded at 3 month time interval. One-Way ANOVA revealed a statistically significant difference among the four time intervals ($P<0.001$) as shown in Table 4. Additionally, in groups II, IV, the highest percentage of canal width decrease was recorded at 12 month time interval while the lowest value was recorded at 3 month time interval. One-Way ANOVA revealed a statistically significant difference among the four compared follow up time intervals ($P<0.001$). Concerning the four groups, Tukey's pair-wise test was done and it recorded statistically significant differences among all compared time intervals ($P\leq 0.001$) as showed in Table 6.

Furthermore, comparison between tested medications regardless of the type of scaffolds and time intervals revealed that mTAP medication showed better results as it recorded higher percentage of canal width decrease compared to the other medication with a statistically significant difference between both tested medications (Fig.7).

When the both tested scaffolds were compared regardless the type of medication and time interval, better results were obtained whenever revascularization was stimulated using PRP scaffold compared to using blood clot scaffold where they recorded percentage of canal width decrease with a statistically significant difference between them as presented in Fig.8.

TABLE (3) Pair-wise statistical analysis of percentage of root length increase among the different follow up time intervals for all tested groups

		TUKEY'S Test					
		P-3M&P-6M	P-3M&P-9M	P-3M&P-12M	P-6M&P-9M	P-6M&P-12M	P-9M&P-12M
% of Root length increase	Group I	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group II	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group IV	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

TABLE (4) Percentage of canal width decrease and its mean ± SD of the four tested groups at each follow up time intervals (immediate postoperative, 3, 6, 9 and 12months) and their statistical analysis.

Canal width	Group I Blood clot/ mTAP		Group II PRP/ mTAP		Group III Blood clot/Ca (OH) ₂ +CHX		Group IV PRP/ Ca (OH) ₂ +CHX		ANOVA	
	Time interval	Mean ± SD	% of Change	Mean ± SD	% of Change	Mean ± SD	% of Change	Mean ± SD	% of Change	P-value
Immediate post		5.139 ±0.488		5.624 ±0.866		5.139 ±0.661		4.589 ±0.837		0.096
Post 3 Months (p-3)		5.139 ±0.488	0.00	5.622 ±0.864	0.02	5.139 ±0.659	0.00	4.587 ±0.837	0.02	0.096 0.579
Post 6 Months (p-6)		5.116 ±0.485	0.48	5.580 ±0.868	0.78	5.114 ±0.663	0.44	4.563 ±0.834	0.57	0.105 0.002*
Post 9 Months (p-9)		5.080 ±0.484	1.14	5.526 ±0.866	1.74	5.091 ±0.663	0.93	4.524 ±0.832	1.42	0.111 <0.001*
Post 12 Months (p-12)		5.047 ±0.485	1.79	5.475 ±0.862	2.64	5.067 ±0.660	1.41	4.474 ±0.832	2.55	0.106 <0.001*
ANOVA P-value		0.996	<0.001*	0.997	<0.001*	1.000	<0.001*	0.999	<0.001*	

TABLE (5) Pair-wise statistical analysis of percentages of canal width decrease of tested groups at 6,9,12 months follow up periods

		TUKEY'S Test					
		I&II	I&III	I&IV	II&III	II&IV	III&IV
% of canal width decrease	P-6M	0.006*	0.787	0.969	0.052*	0.002*	0.520
	P-9M	<0.001*	0.015*	0.002*	0.051*	0.001*	0.052*
	P-12M	<0.001*	<0.001*	0.001*	0.001*	0.001*	0.002*

TABLE (6) Pair-wise statistical analysis of percentages of canal width decrease among four follow up time intervals in all tested groups

		TUKEY'S Test					
		P-3M&P-6M	P-3M&P-9M	P-3M&P-12M	P-6M&P-9M	P-6M&P-12M	P-9M&P-12M
% of Canal width decrease	Group I	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.001*
	Group II	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group IV	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

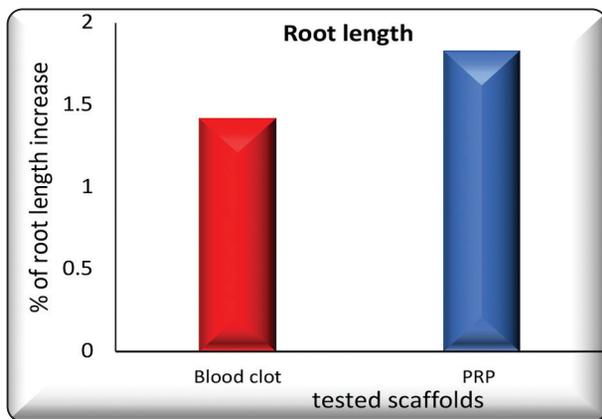


Fig. (7) Bar chart showing percentage of canal width decrease of mTAP medication versus Ca(OH)₂ mixed with 2%CHX regardless the type of scaffolds and time intervals

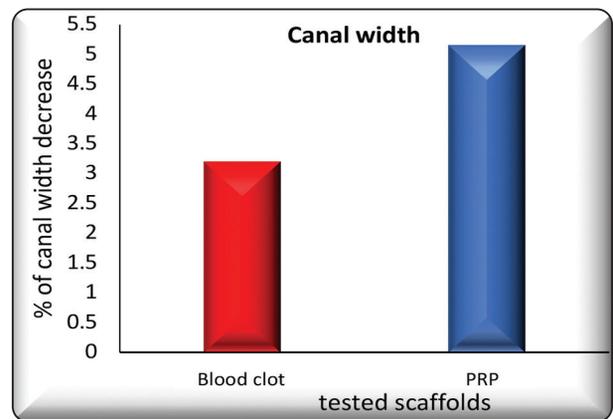


Fig. (8) Bar chart showing percentage of canal width decrease of PRP scaffold versus blood clot scaffold regardless the type of medications and time intervals

Results of apical foramen width decrease:

Comparison of the percentage of apical foramen width decrease among the four tested groups at each follow up interval showed that after the first 3 months follow up, all groups didn't reveal any apical foramen diameter decrease while at 6,9 and 12 months follow up intervals, group II showed the highest apical foramen width decrease value followed by group IV, group I and group III (Table 7). Regarding 9 and 12 months follow up period, Tukey's Pair-wise comparison test showed statistically significant difference between different tested groups as shown in Table 8.

Comparison of percentage of apical foramen

width decrease among different follow up time intervals (3,6,9,12 months in comparison to immediate postoperative) for each tested group was performed (Table 7). All groups didn't reveal any apical foramen diameter change at 3months follow up interval while the four tested groups recorded the highest apical foramen diameter decrease at 12 months followed by 9 and 6 months follow up intervals respectively (Table 7).

Concerning all tested groups, One-Way ANOVA revealed statistically significant differences among the four follow up time intervals. Tukey's pair-wise test recorded statistically significant differences among all tested time intervals ($P \leq 0.05$) as showed in Table 9.

Furthermore, comparison between tested medications regardless of the type of scaffolds and time intervals, revealed that mTAP medication showed better results as it recorded higher percentage of apical foramen diameter decrease compared to the other medication with a statistically significant difference between both tested medications as shown in Fig.9.

When the both tested scaffolds were compared regardless the type of medication and time interval, revascularization using PRP scaffold yielded better results when compared to blood clot scaffold with a statistically significant difference between them ($P \leq 0.001$) as presented in Fig.10.

TABLE (7) Percentage of apical foramen width decrease and its mean \pm SD of the four groups at each follow up time interval (immediate postoperative, 3, 6, 9 and 12months) and their statistical analysis.

Apical foramen width	Group I Blood clot/mTAP		Group II PRP/mTAP		Group III Blood clot/ Ca(OH) ₂ +CHX		Group IV PRP/ Ca(OH) ₂ +CHX		ANOVA	
	Mean \pm SD	% of change	Mean \pm SD	% of change	Mean \pm SD	% of change	Mean \pm SD	% of change	Mean \pm SD	% of change
Immediate post	4.272 \pm 0.439		4.270 \pm 0.716		4.323 \pm 0.547		4.287 \pm 0.443		0.356	
Post 3 Months	4.272 \pm 0.439	0.00	4.270 \pm 0.716	0.00	4.323 \pm 0.547	0.00	4.287 \pm 0.443	0.00	0.356	-
Post 6 Months	4.254 \pm 0.439	0.42	4.247 \pm 0.716	0.53	4.305 \pm 0.544	0.41	4.267 \pm 0.444	0.46	0.265	0.322
Post 9 Months	4.217 \pm 0.441	1.24	4.190 \pm 0.717	1.87	4.273 \pm 0.539	1.15	4.229 \pm 0.442	1.35	0.145	<0.001*
Post 12 Months	4.183 \pm 0.442	2.03	4.139 \pm 0.715	3.06	4.249 \pm 0.539	1.71	4.183 \pm 0.435	2.42	0.133	<0.001*
ANOVA P-alue	0.996	<0.001*	0.997	<0.001*	1.000	<0.001*	0.999	<0.001*		

TABLE (8) Pair-wise statistical analysis of percentage of apical foramen width decrease of tested groups at 9 and 12 months follow up time intervals

		TUKEY'S Test					
		I&II	I&III	I&IV	II&III	II&IV	III&IV
% of apical foramen width decrease	P-9M	<0.001*	0.008*	0.145	0.006*	0.027*	0.024*
	P-12M	<0.001*	<0.001*	0.034*	0.032*	0.030*	0.031*

TABLE (9) Pair-wise statistical analysis of percentage of apical foramen decrease at four follow up time intervals in all tested groups

		TUKEY'S Test					
		P-3M&P-6M	P-3M&P-9M	P-3M&P-12M	P-6M&P-9M	P-6M&P-12M	P-9M&P-12M
% of apical foramen width decrease	Group I	0.014*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group II	0.011*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group III	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
	Group IV	0.019*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

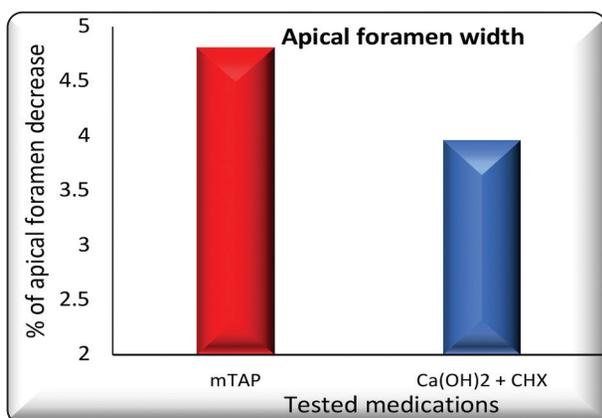


Fig. (9) Bar chart showing percentage of apical foramen diameter decrease of mTAP medication versus Ca(OH)₂ mixed with 2%CHX regardless the type of scaffolds and time intervals

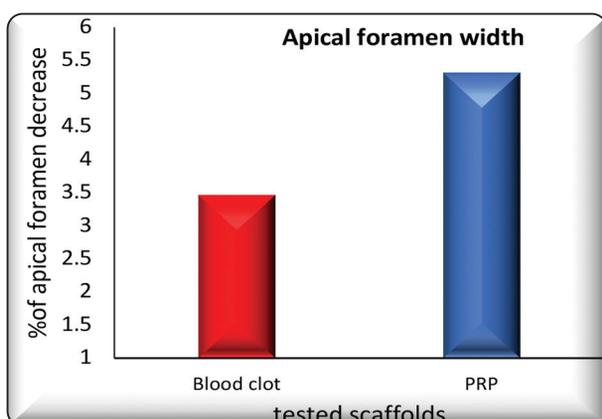


Fig. (10) Bar chart showing percentage of apical foramen diameter decrease of PRP scaffold versus blood clot scaffold regardless the type of medications and time intervals

DISCUSSION

Success of conventional endodontic treatment depends mainly on chemo-mechanical preparation of root canals to eliminate any infection. However, the treatment phase is more complicated in cases of immature teeth with open apices and fragile root canal walls due to decreased thickness of dentin that is more liable to fracture. In addition, the configuration of the open apex makes it difficult to perform complete obturation of the root canal with increasing the risk of over extension of filling material into the periapical area ^[12].

Traditional treatment modalities, Ca(OH)₂ and MTA apexification, don't allow continued root development and don't solve the problem of thin brittle roots ^[13]. Therefore, this study was performed to radiographically and clinically evaluate revascularization induced maturogenesis of non-vital immature teeth.

In the present study, Patients with immature upper central incisors were selected because their early presence in the oral cavity cause them to be the most common teeth subjected to caries or trauma before complete root formation ^[14].

Since elimination of bacteria from the root canal space is mandatory to achieve successful revascularization [15], irrigation protocol was done by using 20 mL of 1.5% sterile NaOCl solution which is thought to be enough to achieve satisfying bacterial elimination with preservation of remnant vital apical tissues ^[16]. This was followed by 20 mL of 17% EDTA solution as it is responsible for releasing of growth factors from dentin, thereby increasing their bioavailability. These growth factors stimulate stem cell proliferation and differentiation ^[16]. An intermediate rinse of sterile saline was used in the present study to minimize erosion effect on dentin that may develop if EDTA was used directly after NaOCl. Irrigants were delivered using 30-gauge side-vented needle adjusted at 3mm shorter than the working length to avoid injury of vital remnant periapical cells to increase the chance of their proliferation and differentiation into more specific hard tissue laying cells either odontoblasts or cementoblasts resulting in continuity of root formation and successful revascularization would occur ^[16].

The intra-canal medicaments used in this study were either mTAP or Ca(OH)₂ mixed with 2% CHX. In cases treated using mTAP, doxycycline was replaced by cefaclor to avoid its discoloration effect on the crown of the tooth ^[5]. Combination of the three antibiotics was proved to be enough to eliminate bacteria from infected root canals ^[17].

However, other studies found that combination of antibiotics had minimal inhibitory effect against *E. faecalis* and *E. faecium* presented within root canal [18,19]. In addition, it was found that some root canal bacteria have developed antibiotic resistance. Another risk of antibiotic combination is allergic reaction in sensitive patient [18]. All these facts created controversy about the usage of combination of antibiotics as intracanal medication, especially in revascularization [19].

Moreover, Ca(OH)_2 mixed with 2% CHX was used as a medication in this study where Ca(OH)_2 is used mainly due its favorable antimicrobial action because of its high alkaline pH (12.5-12.8) in addition to its ability to dissolve necrotic tissue in the root canal. However, Ca(OH)_2 alone is not effective against all types of bacteria present in root canal and its high pH is potentially dangerous to bacterial as well as human cells that may affect preservation of remnant vital tissue which is required for revascularization [18]. On the other hand, CHX is characterized by its substantivity, biocompatibility and wide antimicrobial activity especially against microorganisms which are resistant to Ca(OH)_2 as *E. faecalis* [20]. Some studies found that mixing Ca(OH)_2 with 2% CHX does not improve its antibacterial property as an intracanal medicament against *E. faecalis* that is essential for disinfection of root canal space which is critical for regeneration of new tissue resulting in successful revascularization [21]. So, mixing Ca(OH)_2 with CHX to improve its antibacterial property as an intracanal medicament in elimination of *E. faecalis* remains a matter of controversy [22].

Blood clot was used as a scaffold utilizing its cross-linked fibrin matrix within the clot to trap cells necessary for tissue regeneration and provided a suitable pathway for cells from the periapical area to migrate into the root canal [23]. PRP was used as a controlled scaffold to replace the blood clot in tissue regeneration. Recently PRP has shown its ability to improve regeneration and cell proliferation [24].

It has additional several advantages such as easier preparation and application, more hardness, and more suitability for the subsequent placement of MTA and permanent restorations. In addition, anaesthesia is not necessary since periapical bleeding is not indicated and PRP can be used in patients where bleeding into root canal cannot be performed as in teeth with large epithelialized periapical lesions [25]. Unfortunately, there is a wide controversy about using blood clot or PRP as a scaffold. Some studies suggested that PRP is a better scaffold than blood clot especially in cases with epithelialized periapical lesions with more controlled outcomes rather than unfavorable ones, as root canal obliteration, which is more associated with using of blood clot as a scaffold [26,27] while other studies found that PRP yielded nearly the same results similar to blood clot without the need of extra equipment or further steps especially in young age patients [28,29]. Hence, this study compared PRP to blood clot as scaffolds.

Coronal seal was achieved through usage of MTA as orifice plug to seal the orifice and the coronal 3mm of the canal which is a suitable sufficient thickness of MTA able to create tight seal that ensure a bacteria free environment throughout the root canal [30]. In addition, composite resin was used as a final coronal restoration to ensure sealability, prevent leakage, prevent the risk of coronal failure of revascularization and provide better esthetics for patient satisfaction [30].

Concerning the results of the present study, on clinical point of view, the four groups showed successful results where patients were completely asymptomatic throughout the study period with no tenderness to palpation or percussion and without any crown discoloration. No preoperative or postoperative swelling was observed in included patients. In addition, sinus tract which was found in two cases related to group I and II were completely resolved after three months of follow up since the main line of treatment of sinus tract is elimination of the source infection which in these cases was

done through disinfection of the root canal with NaOCl and EDTA in addition to mTAP intracanal medication in addition to physical occupation of the root canal space using either blood clot or PRP scaffold which prevented further infection development^[31].

Radiographically, the four groups achieved successful revascularization which was expressed as improvements in root length, canal width decrease and apical foramen width decrease with statistically significant differences among tested groups especially at 9 and 12 months follow up periods. These results indicated that revascularization provided successful management technique for necrotic immature teeth which can be explained by multiple different possible explanations.

The first one; two types of cells are required to achieve a normal root development: odontoblasts and epithelial cells of Hertwig's sheath which present in abundance in the apical area of immature teeth and are able to resist inflammation^[32,33]. In addition, the remnant vital pulp cells at the apical end of root canal are able to proliferate and differentiate into secondary odontoblasts that will generate dentin on root canal walls and thus allow root maturation guided by Hertwig's intact epithelial root sheath leading to a successful revascularization^[32].

Another explanation may be related to the action of periodontal ligament stem cells [34,35] especially in case of the destruction of apical papilla cells. These cells could proliferate and differentiate into cementoblasts or osteoblasts and grow within the open apex of the root and lay down cementum-like or bone-like hard tissue continuous with the cellular cementum at the apical end of the tooth leading to completion of the root and success of revascularization^[36].

These findings were supported by Trope^[37] in 2008 who demonstrated that clinical and radiographic healing could be observed after 22 days. The authors applied the revascularization technique in a lower right second premolar with

open apex, with clinical and radiographic aspects of apical periodontitis using blood clot as scaffold and digital periapical radiographs for evaluation.

Additionally, a study of Pramila and Muthu^[38], concluded that under certain circumstances including disinfection using NaOCl, TAP intracanal medication, blood clot scaffold and proper coronal sealing using MTA followed by glass ionomer filling material, teeth with necrotic pulps and open apices can regenerate pulp tissue, encourage root growth and complete apex formation. Furthermore, Kim *et al.*,^[25] performed a study in which revascularization attempt was applied using TAP as intracanal medication and blood clot as a scaffold and found that patients were asymptomatic and showed apical periodontitis resolution at the end of the treatment.

The current results revealed that blood clot as a scaffold presented satisfying results. This may be due to the effect of instrumentation beyond the limits of the apical foramen into the periapical area in order to induce bleeding. This may cause transportation of mesenchymal stem cells derived from the bone marrow or from the apical papilla into the canal lumen, giving rise to bone or dentin-like tissues which increase the chance of increasing root length^[39,40].

In addition, blood clot scaffold is rich in growth factors (TGF, PDGF and VEGF) that facilitates the differentiation, growth, and maturation of fibroblasts, odontoblasts, and cementoblasts from their undifferentiated precursors^[39,41,42].

However, in this study, PRP has achieved better results as a scaffold compared to blood clot. PRP can form a three dimensional fibrin matrix that helps entrap the growth factors. Additionally, platelet concentration in PRP exceeds 1 million/mL, which is 5 times more than that of the normal platelet count found in blood clot [38,43], which secrete seven fundamental protein growth factors (PDGF, TGF, VEGF, EGF, FGF, CTGF and IGF-1) that increased the cell proliferation over time

when compared to the blood clot. Moreover, these secreted growth factors direct matrix formation, osteoid tissue production and collagen synthesis which induce tissue regeneration or repair as they stimulate the migration of stem cells present in the apical tissues: vital pulp cells, periodontal ligament cells, ADPCs, bone marrow cells and even from periapical lesions^[44,45].

In addition, it is relatively faster to create vital tissue in the root canal compared to blood clot. Moreover, there is limitation of the blood clot as a scaffold for pulp regeneration since the composition of a clot is variable, and concentrations of cells trapped in a clot might vary leading to unpredictable outcomes which explains superior results of PRP as a scaffold^[46].

The obtained results from this study agreed with Nagata *et al.*,^[8] where the authors concluded that revascularization using PRP can potentially improve and accelerate the desired biological result of regenerative technique. Furthermore, Turkey *et al.*,^[9] agreed with the obtained results where they concluded that PRP can act as an excellent scaffold for regenerative endodontic procedures with better results than blood clot.

Unlikely, the present results disagreed with that of Alagl *et al.*,^[47] where they concluded that PRP was not significantly different from blood clot as a scaffold. The differences may be attributed to the different type of teeth involved in the study where they evaluated non-vital upper and lower immature premolars in addition to non-vital immature upper central incisors. Similarly, ElSheshtawy *et al.*,^[48] concluded that both blood clot and PRP scaffolds showed comparable results. This may be referred to the different methodology where they added collagen plug to both scaffolds.

Regarding the used intracanal medications, mTAP had better results compared to $\text{Ca}(\text{OH})_2$ mixed with 2% CHX. This may be attributed to the

difference in pH of CHX and $\text{Ca}(\text{OH})_2$ where pH of CHX ranges from 5.5 to 7 while the pH of $\text{Ca}(\text{OH})_2$ ranges from 12.5 to 12.8. This difference may lead to reduced antibacterial effect of CHX due to its deprotonation at high pH which means removal of a proton or hydrogen cation from the acid in an acid–base reaction leading to formation of the conjugate base of that acid, which in turn reduces its solubility and alters its interaction with bacterial surfaces as a result of the altered charge of its molecule^[49].

Moreover, binding of CHX molecule to $\text{Ca}(\text{OH})_2$ ions inhibits the free release of CHX molecule which in turn decreased its substantivity, affecting one of the important advantages for its use, and reducing its time of antibacterial action which in turn affects its antimicrobial power [38]. The reduced antibacterial activity affect the revascularization success as improper elimination of bacteria from the root canal interfere with the proper environment that should be provided within the root canal to allow migration, attachment and proliferation of mesenchymal stem cells either through the physical occupancy by bacteria itself or their byproducts that would inhibit the activity of mesenchymal cells which in turn would affect the success rate of revascularization^[38]. In addition, CHX has a direct cytotoxic effect on human stem cells which can be explained by its lytic properties on cell content or glutathione depletion which is an important substance involved in tissue building and repair impairing the success of revascularization^[50].

Furthermore, literature revealed that $\text{Ca}(\text{OH})_2$ has destructive as well as inductive properties as it can solubilize bioactive molecules, including growth factors of human dentin matrix, which is responsible for stimulation of mesenchymal pulp cells to differentiate into odontoblast cells. In addition, $\text{Ca}(\text{OH})_2$ could damage epithelial cell rests of Malassez that is essential for multipotent stem cell proliferation resulting in impaired success of revascularization^[22].

On the other hand, mTAP can help promote functional development of the pulp–dentin complex as metronidazole and ciprofloxacin can induce the formation of fibroblasts and secondary odontoblasts through modulation of inflammatory mediators and growth factors that would control the differentiation rate of mesenchymal stem cells [51]. In addition, the poly antibiotics use allow better antibacterial effect through deeper penetration into dentinal tubules and better disruption of bacterial biofilms compared to the mix of $\text{Ca}(\text{OH})_2$ and 2% CHX¹⁷⁶ explaining its better results.

Aggarwal et al., [52] concluded that the TAP creamy paste resulted in better apical healing when compared to $\text{Ca}(\text{OH})_2$ which coincide with the obtained results. In addition, Bains *et al.*, [53] supported the results obtained in this study as they assumed that there were significantly greater number of canals negative for bacteria in the TAP antibiotic group than $\text{Ca}(\text{OH})_2$ plus 2% CHX group.

In contrary, Brogni *et al.*, [54] disagreed with these findings as they concluded that the association between 2% CHX and $\text{Ca}(\text{OH})_2$ as an intra-canal dressing showed more favorable results than TAP. The different results can be attributed to the different inclusion criteria of the selected cases in which they used revascularization procedure as a retreatment procedure after failure of first attempt of endodontic treatment in addition to the different composition of TAP in which minocycline was used instead of cefaclor.

CONCLUSIONS

PRP has great potential to induce the growth of immature necrotic permanent teeth as compared to blood clot and mTAP can achieve superior maturogenesis criteria of immature necrotic permanent teeth compared to $\text{Ca}(\text{OH})_2$ mixed with CHX concerning root length increase, canal width decrease and apical foramen decrease.

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