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Evaluation of Removal of DAP Intra-canal Medication Mixed with Different Vehicles from Immature Root Canals using XP Endo Finisher

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ORIGINAL STUDY

Evaluation of Removal of Double Antibiotic Paste Intra-canal Medication Mixed with Different Vehicles From Immature Root Canals Using XP Endo Finisher

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Abstract

Purpose: The present study was performed to evaluate the removal of double antibiotics paste (DAP) intra-canal medication mixed with different vehicles from immature root canals. **Patients and methods:** Thirty-seven single-rooted teeth were prepared using ProTaper universal till size F5. Then, canals were allocated into three groups according to the type of vehicle that was used for mixing DAP. Each group comprises 11 samples, four of which were assigned as the control group. Group I: root canal filled with DAP mixed with polyethylene glycol (PEG400), group II: root canal filled with DAP mixed with chitosan, and group III: root canal filled with DAP mixed with distilled water. Samples were stored at 37 °C for 3 weeks under a humid environment, followed by removal of DAP via XP-Endo Finisher file by continuous irrigation with 2.6% NaOCl then 17% EDTA and finally flushed with distilled water. All samples were sectioned longitudinally into two halves. Each half was photographed, and the image was digitized to calculate the percentage of the remaining amount of DAP at each third (statistical tests and level of significance). **Results:** XP-Endo Finisher removed significantly more DAP mixed with PEG400 than other groups ($P < 0.05$). A significant difference between groups, especially between the control group (the highest remnant percentage) and PEG 400 group (the lowest remnant percentage). **Conclusion:** From the overall mean for every group, we can conclude that the PEG 400 as the vehicle was (the lowest in remnant percentage), especially in the apical third.

Keywords: Double antibiotic past, Polyethylene glycol 400, XP endo finisher

1. Introduction

One of the most important sequelae that face young patients following the loss of dental pulp vitality is the arrest of root formation and maturation. Thin funnel-shaped walls of the root canals and widely opened apices of the roots are common features of this situation which in turn can lead to serious obstacles during endodontic treatment procedures such as inadequately debrided root canal, inaccurate obturation or root fracture in immature teeth [1].

A newly introduced treatment option for teeth affected by necrosis with an open apex is known as the regenerative endodontic procedure (REP). It is defined as 'biologically based procedures formulated to substitute the injured tissues' as dentin and roots, as well as dentin-pulpal complex cells. REP aims to introduce an appropriate condition in the root canal to encourage the re-population of the root canal by undifferentiated mesenchymal cells. Besides, it provokes pulp and periapical tissue recovery, encourages continual development of the roots, the added thickness of the root canal walls, and narrowing and closure of the apex [2].

The most important element influencing the outcome of revascularization and is root canal space disinfection using irrigation and intra-canal medication. Different intra-canal medicinal agents and products have been utilized to disinfect the root

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canals in the REP. Namely, triple antibiotic paste (TAP) with different combinations, double antibiotic paste (DAP), and calcium hydroxide (CH). CH is a commonly used intra-canal therapeutic agent to disinfect the root canals between appointments in the REP. It has biocompatibility, results in suppression of the action of osteoclasts, and has regenerative features [3].

Another type of intra-canal therapeutic agent is antibiotic pastes. Particularly, TAP (which comprises minocycline, ciprofloxacin, as well as metronidazole), or DAP (which comprises ciprofloxacin, as well as metronidazole), Both are widely utilized in cases where CH could not relieve the adverse conditions and symptoms [4]. Both TAP and DAP had detrimental effects on human stem cells in the apical papilla with higher concentrations. Thus, these pastes should be removed completely from root canals to inhibit their adverse effects on these cells [5].

Their handling characteristics have been promoted by the incorporation of vehicles, which in turn improved their diffusion within dentinal tubules, antibacterial features, and release [6]. Various types of vehicles can be mixed with DAP: water-based soluble (distilled water, saline solution, and chlorhexidine), non-water-based soluble (propylene glycol and polyethylene glycol (PEG)) [7] as well as (chitosan).

Polyethylene glycol (PEG 400) is a colorless, water-soluble, and hygroscopic polymer that is miscible with water in all proportions, so it can be used as a solvent in its own right or can be mixed with water. PEG is classified as 'Generally Recognized as Safe' and has high biocompatibility materials based on PEG solvents that can achieve a pH which is above 12.4−12.6, the nominal limit for aqueous compositions [8].

Chitosan: Water-soluble polymers are explored as vehicles for biomolecules on sustained release strategies. Chitosan has gained interest because it is a biodegradable, biocompatible, and low-cost polymer of easy manipulation for developing hydrogels or pastes [9]. The structure of chitosan includes NH_2 groups thus when the polymer is in an acidic medium protonation occurs and chitosan behaves as a polyelectrolyte that forms a viscous jelly matrix when dissolved in concentrated acidic medium [10]. It has been used extensively in the pharmaceutical industry due to its biocompatibility and the additional advantage of slow and controlled drug release. For these reasons, it has been recently used as a carrier for intra-canal medicament in dentistry [11].

It is strongly advised to recapitulate the root canal using a master apical file at the exact working length and irrigate vigorously with NaOCL and EDTA, to remove all the remnants of the intra-canal medicament [12]. Recently XP-Endo Finisher (XEF) was presented with promising root canal cleaning and disinfection results [13]. Previous studies have demonstrated promising results using the XEF to remove debris and the smear layer [14−20]. It relies on the shape memory feature of the NiTi alloy, in addition to a tiny core, size 25 and no taper, to be utilized next to root canal debridement with size 25 or more to clean extremely complicated anatomy and regions of difficult access, in addition to the elimination of intra-canal medication [21].

There is limited knowledge about the influence of vehicles on the removal of DAP from the root canal wall. Hence, the current research aims to study the DAP removal capacity when associated with different vehicles. The null hypothesis was there is no effect in the remnant percentage of DAP between different vehicles.

2. Patients and methods

2.1. Sample size calculation

The present study was performed to evaluate the removal of DAP mixed with three vehicles from root canals by Digital camera (Canon EOS 750 D, Taiwan, Japan, 100 mm macro lens f/2.8). A minimum total sample size of 37 samples was enough to ascertain an effect size of 0.35 regarding the previous study [22]. The power of the study was set at 0.95 and a significance level ($P < 0.05$) and partial squared eta of 0.73. According to sample size calculations, each group (G1, G2, G3) was represented by a minimum of 11 samples. Two samples without DAP were used as negative controls, and two samples filled with DAP (without removal of paste) were used as positive controls. The sample size was calculated according to G*Power software version 3.1.9.3. f: is the effect size; $\alpha = 0.05$; $\beta = 0.05$, 0.20, Power = 1- β.

2.2. Samples selection

Thirty-seven freshly extracted, single-rooted human bicuspids were chosen for the current study. By using periapical radiographs and stereomicroscope, the teeth exhibited the following criteria were included; free from caries, cracks, endodontic treatment, curved root canals, internal resorption, or calcification. Ethical approval for the use of extracted human teeth was obtained by guidelines from the Research Ethics Committee (REC) of the faculty of Dental Medicine for Giles Al-Azhar University (REC-CL-23-07). Preoperative periapical radiographs were taken for all teeth in both buccolingual

and mesiodistal directions to confirm the selected criteria. The selected teeth were rinsed under tap water and immersed into 2.6% sodium hypochlorite solution for 30 min to remove any soft tissue covering; any hard deposits attached to the teeth were cleaned using an ultrasonic scaler, then stored in a jar containing normal saline solution at room temperature till its usage.

2.3. Samples preparation

Removal of the apex and standardization of the centralization of the canal exit was done. Endo Z bur was utilized to remove 3 mm of the root tip. The bur was held at 90° with the long axis of each tooth. This procedure was performed under copious water coolant. While a rose-head bur was utilized to prepare the coronal access cavity. In addition, a K file of size 15 was placed inside the canal to check canal patency. The procedure was performed at a working length that was specified after subtracting 1 mm from the size 15 file whenever visible from the canal exit in each tooth. The root canal preparation was performed with the aid of rotary instrumentation Protaper Rotary file system (Dentsply Maillefer, Ballaigues, Switzerland) till size (F5) via the endodontic micromotor Geo Soft (Geosoft Endoest Motor Mini- Russian- Speed 200 rpm−600 rpm and Torque 0.2−3.5 Ncm). To mimic teeth with immature apices, peso reamers (Dentsply, Washington, USA) between #1 to #6 were introduced in the root canals by using a low-speed motor, and a #6 peso reamer was allowed to protrude 1 mm beyond the apex to obtain the apical diameter of 1.7 mm. Irrigation was done by 3 ml of 2.6% NaOCl after each instrument using a syringe and a 27-gauge side vented needle. The final irrigation of canals was done using saline which was followed by paper points drying [23].

2.4. Preparation of double antibiotic past

To prepare the concentration of DAP (1 gm/ml). One tablet of Ciprocin (ciprofloxacin 500 mg-BioFarma, Istanbul, Turkey) and one tablet of Flagyl (metronidazole 500 mg-Sanofi, Istanbul, Turkey) was crushed and ground into a fine powder using a mortar and pestle. Then, a weigh by digital laboratory scale (Sartorius GL2241-1 S scale 1100 ct- Sachi tools) equivalent to 50 mg of each antibiotic was dissolved in 1 ml of PEG400 (0.05%- LOBA Chemie PVT.LTD.India)-(PEG400 indicates that the average molecular weight of the specific PEG is 400) or chitosan (0.2%- Sigma Co. Egypt, Cairo, Egypt) or distilled water (Ostuka pharm, Egypt) [24].

2.5. Preparation of the chitosan

Chitosan solution was prepared by adding 0.2% gm of 90% deacetyled-chitosan which was dissolved in 100 ml of acetic acid 1%. Stirring of the mixture was done by a magnetic stirrer for 2 h. The solution was stored in a tightly sealed container and used within 2 weeks after preparation and saved in the refrigerator [25].

2.6. Sample grouping

Thirty-seven samples were divided into three main experimental groups (11 for each group) according to the type of vehicle that was used for mixing DAP. Two Samples acted as a negative control (that were not filled with DAP), and two samples as a positive control (that were filled by DAP but not removed). Group I (n = 11) Root canals were filled with DAP mixed with PEG400. Group II (n = 11) Root canals were filled with DAP mixed with chitosan. Group III (n = 11) Root canals were filled with DAP mixed with distilled water.

2.7. Medicament application

The medicaments were delivered through the root canals until they appeared from the apex and up to the cervical line by using a #40 Lentulo spiral at 900 rpm, the root canals of each treatment group were sealed apically with temporary filling material to prevent the pastes from dissolving due to humidity during the storage period and access openings were sealed with glass ionomer, each sample was stored in Eppendorf tube filled with saline till a level of CEJ. The samples were stored at 37 °C with 100% humidity for 3 weeks. The solution was changed every week to avoid dehydration of the sample [26].

2.8. Removal of DAP

An XEF (FKG Dentaire, La Chaux de Fonds, Switzerland) was used to remove DAP from each sample, after 21 days. The rubber stopper was set at one millimeter shorter than the working length. The endodontic micromotor was adjusted at a revolution speed of 800 rpm and a torque of 1 Ncm. The procedure was done in 1 min with an up-down movement and copious irrigation with 20 ml of 2.6% NaOCl. This was followed by a final flush using 20 ml of 17% EDTA using a syringe with a 27-gauge side vented needle. Finally, the root canal was flushed with 5 ml of distilled water [27].

2.9. Root specimen preparation

After the removal of DAP, all samples were fixed on a bench vice, grooved longitudinally in a buccolingual direction using a double-sided diamond disc 7020 (KG Sorensen, Bauru, SP, Brazil) fixed on a low-speed straight handpiece, under water coolant, conserving the inner shelf of dentin surrounding the canal, and each sample was split into mesial and distal halves using a bone hammer or spatula [28]. The residual amount of intra-canal medicament was measured.

2.10. Method of evaluation

2.10.1. The amount of remaining filling material on the root canal walls

All samples were grooved labio-lingually and split into two halves by a diamond disc. Imaging of each half of longitudinal sections by a high-resolution Camera (Canon EOS 750 D, Taiwan, Japan, 100 mm macro lens f/2.8) at a magnification of 10x. Images were transferred to computer system software for image analysis to remnants of the DAP by coloring effect then were transferred (Image J) to calculate the mean percent of the remaining filling materials at all sample lengths and each third (cervical, middle and apical) [27] (Fig. 1).

2.10.2. Photo analysis

The Digital images were processed using the photographic editing software Mountains Map-v (Besancom, France and Adobe Photoshop 7.0, Adobe system Inc, San Jose, California, USA) then the stained area was calculated as % of the total third area using ImageJ software (Version 1.53a National Institutes of Health, USA). The image analysis steps and measurement technique can be summarized in [27] (Fig. 2).

Step 1: Photoshop software was used for the segmentation of each tooth by its outline, using the semiautomatic outline selection tool, in that way. The root canal was isolated from the rest the image and divided into three thirds (cervical, middle, and apical), then using mountain map software the color range of remnants DAP was detected. After that, the areas with remnants DAP (whitish) were subsequently identified and highlighted with blue color, and then separated from the rest of the image.

Step 2: Using Image J Software, the entire visible third area (cervical, middle, or apical) was automatically measured in pixels. From the images of isolated remnants of DAP which were separated in step 1 and applying a threshold, the stained area in each third was automatically measured in Pixels, and then calculated as % of the total third area. Using the following equation:

$$Remnant\ of\ the\ DAP\ \% = \frac{Sum.\ of\ blue\ stained\ area\ (\ pixels)}{Total\ third\ area\ (pixels)} x100$$

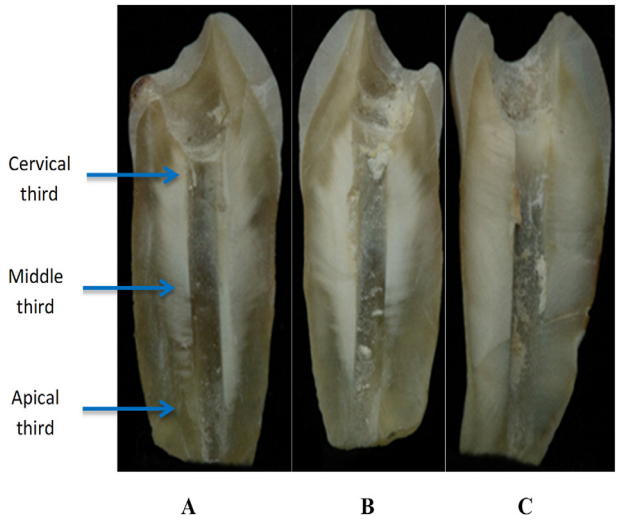


Fig. 1. Photograph represents remnant of the double antibiotic paste in the cervical, middle and apical. A; group I (PEG400), B; group II (chitosan) and C; group III (distilled water).

2.11. Statistical analysis

Statistical analyses for results were performed by applying the analysis of variance test (one and two ways) followed by the *post hoc* test for intra and inter group comparisons. *P* value less than or equal to 0.05 was considered statistically significant (95% significance level). Levene's test was used to test the normality of data. All data were analyzed using the statistical software SPSS (version 25, IBM Co. USA).

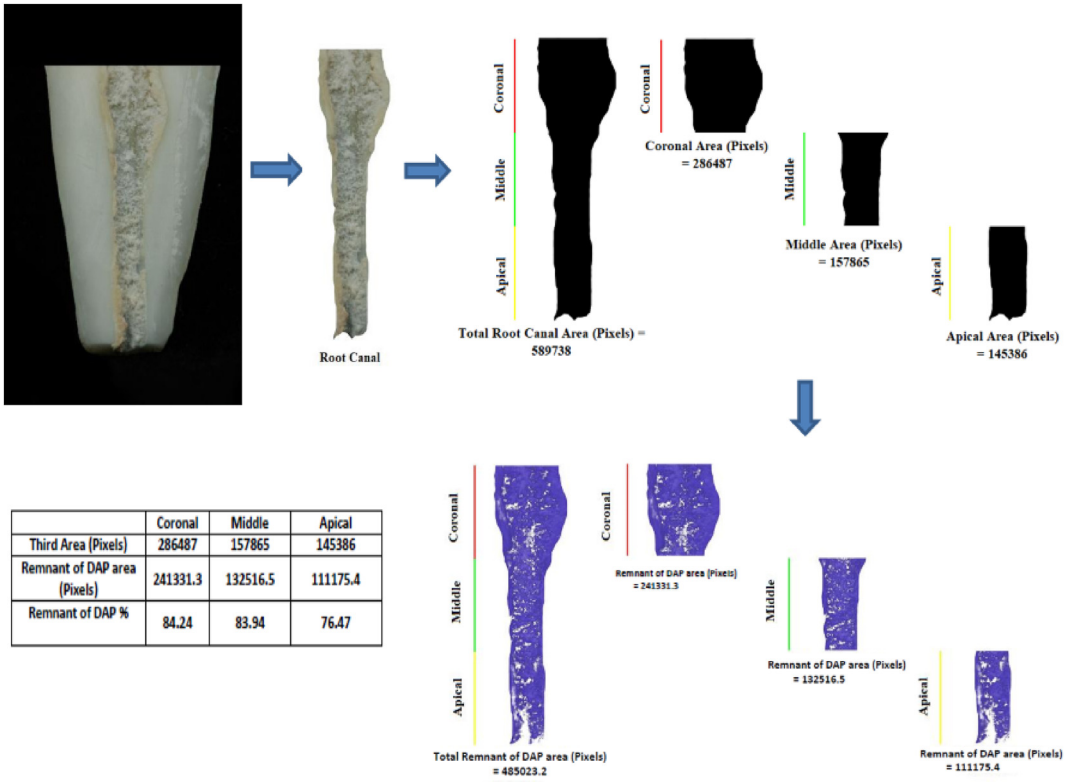
3. Results

Mean values and standard deviation of the percentages of DAP remnants in different thirds (the coronal, the middle, and the apical) among different groups, Table 1.

The comparison between the three groups:

(i) Intra-group comparison"

(a) In the PEG 400 (group I): the percentage of remnant represents (19.63 ± 6.75%) of the cervical third, (7.83 ± 2.86%) of the middle third, and (4.20 ± 1.51%) of the apical third Table 1. There was a statistically significant difference in the mean remnant percentage



	Coronal	Middle	Apical
Third Area (Pixels)	286487	157865	145386
Remnant of DAP area (Pixels)	241331.3	132516.5	111175.4
Remnant of DAP %	84.24	83.94	76.47

Fig. 2. A schematic presentation illustrates the measurement procedure. For example, Sample 1 from the positive control group.

between the three-thirds (P value < 0.05), with the cervical third representing the highest remnant percentage and the apical third viewing the lowest remnant percentage.

(b) In the Chitosan (group II): the percentage of remnant represents (20.78 ± 7.09%) of the cervical third, and (12.37 ± 5.06%) of the middle third, and (9.56 ± 3.52%) of the apical third Fig. 3. Similar to PEG400, there was a statistically significant difference in the mean remnant percentage between the three-thirds (P value < 0.05), with the cervical third representing the highest remnant percentage and the apical third viewing the lowest remnant percentage.

(c) In the distilled water (group III): the percentage of remnant represents (24.87 ± 6.68%) of the cervical third, (16.39 ± 7.24%) of the middle third, and (13.56 ± 2.39%) of the apical third. A similar trend as the above two groups, there was a statistically significant difference in the mean remnant percentage between the three-thirds (P value < 0.05), with the cervical third representing the highest remnant percentage and the apical third viewing the lowest remnant percentage. Fig. 3. In the control group, there was no significant difference in the mean remnant percentage between the three-thirds and the overall P value greater than 0.05.

(ii) Intergroup comparison:

In cervical and middle thirds for each group: The distilled water group achieved the highest mean remnant percentage, and PEG 400 achieved the lowest one. Notwithstanding

Table 1. Comparative evaluation of the mean percentage of remnants in different thirds between all groups.

	Cervical	Middle	Apical	P value intragroup comparison
Group I (PEG400)	$19.63 \pm 6.75^{a\ X}$	7.83 ± 2.86^{bX}	4.20 ± 1.51^{bZ}	0.000^{S}
Group II (Chitosan)	20.78 ± 7.09^{aX}	12.37 ± 5.06^{bX}	9.56 ± 3.52^{bX}	0.000^{S}
Group III (Distilled water)	24.87 ± 6.68^{aX}	16.39 ± 7.24^{bX}	13.56 ± 2.39^{bY}	0.031^{S}
Positive control group	98.04 ± 2.06^{aW}	99.8 ± 3.21^{aW}	97.85 ± 2.19^{aW}	0.833^{NS}
P value intergroup comparison	0.001^{S}	0.000^{S}	0.000^{S}	

Capital letters for inter-group comparison and small letters for intra-group comparison, and the means with different superscripts are statistically significant different at P less than or equal to 0.05 (Tukey post hoc test). S = statistically significant ($P \leq 0.05$) NS= Nonsignificant P value greater than 0.05.

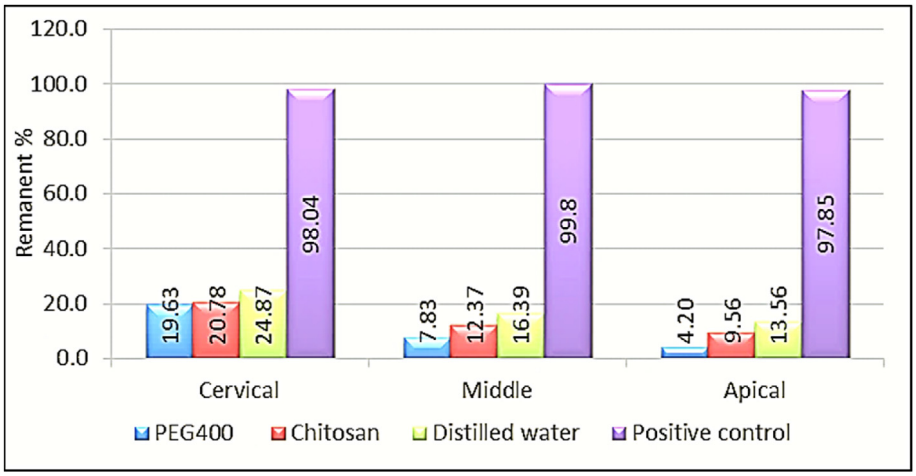


Fig. 3. Column chart representing mean percentage of remnant of the double antibiotic paste in different thirds (cervical, middle, apical) between all groups (PEG400, Chitosan, Distilled water, positive control).

that, there is no statistically significant difference between PEG 400, chitosan, and water groups Table 1.

(a) In the apical third: The control group achieved the highest mean remnant percentage, and PEG 400 achieved the lowest one. There is a statistically significant difference between all groups (P value < 0.05).

From the overall mean for every group. The control group showed the highest significant percentage of the remnant (P value < 0.05).

4. Discussion

The main objective of endodontic regeneration is the restoration of the necrotic nonvital immature teeth to a healthy state, provocation of further development of roots, and strengthening of dentinal walls by the deposition of hard dental tissues. The use of an appropriate dental biomaterial, utilization of stem cells, and applying all the strict infection control measures are considered as the 'pulp tissue engineering triad' that greatly influences the success of endodontic regeneration procedures [29].

Little or no instrumentation is required during the application of regenerative endodontic treatment procedures, as instrumentation may result in the weakening of the already weak and thin immature roots [30]. Hence, the usage of irrigating solutions and intra-canal medications is very essential to eliminate pathogens as well as improve the biological habitat for the REP [31].

Various biomaterials have been utilized for intra-canal medication such as TAP which is considered the first material of choice due to its significant enhancement of the development of thicker root walls in comparison to other intra-canal medicaments. Ciprofloxacin, metronidazole, and minocycline are the three main components of TAP. Although the positive effects of minocycline on modulating host responses, it chelates calcium ions and results in the formation of an unfavorable chelation product that causes hard tissue discoloration. Hence, DAP, which is composed of metronidazole and ciprofloxacin only, is strongly recommended in regenerative endodontics to overcome the adverse effects of minocycline-containing antibiotic pastes [32].

On the other hand, DAP has a demineralizing effect which is greatly important in establishing all favorable conditions for undifferentiated stem cells to attach to the root canal surface, and exposing collagen fibers as well as different growth factors. However, root dentin should not be exposed to increased concentrations of DAP as it may lead to root fractures due to excessive demineralization by the acidic antibiotic mixtures [27].

Clinical application of TAP and DAP is usually performed at a concentration of 1000 mg/ml which is sufficient to change dentine and prevent the survival of SCAP [33]. The American Association of Endodontists (AAE Clinical Considerations for a Regenerative Procedure recommended the use of diluted antibiotics in a concentration of 0.1–1 mg/ml for endodontic regeneration procedures [34].

The DAP can be associated with different vehicles because these vehicles may affect several properties of endodontic materials, such as flow, setting time, calcium ion release, pH, the ability to induce mineralized tissue formation, and biocompatibility [35]. Various kinds of vehicles have been introduced to be utilized in conjunction with intra-canal medicaments, such as: soluble aqueous (distilled water, saline solution, and chlorhexidine); soluble

nonaqueous (propylene glycol and PEG) [7], and (chitosan) [36].

The aim of simulation of clinical conditions and the natural structure of dentin, natural teeth has been used in the current study instead of artificial ones [37]. Single-canal teeth were used to reduce the influence of variations among different teeth samples including root canal system complexity and the common existence of lateral and accessory canals that might lead to difficulty in root canal debridement [4].

The pastes were placed inside the root canals with the aid of a lentulo spiral until the medicaments were advent at the apical foramen and packed to the working length. DAP remained inside the canals for three weeks to simulate clinical conditions [21].

The presence of DAP remnants attached to the internal dentinal surface of canals could negatively influence the successful long-term outcomes of the whole procedure. Hence, their vigorous and complete removal is mandatory. In the current methodology, the ability of XPF to eliminate DAP mixed with different vehicles (PEG400, Chitosan, and distilled water) out of the immature straight root canals was investigated.

The current study showed that the least amount of remnants was observed in the apical third of all groups. This finding agrees with previous studies which concluded that the XPF provided few amounts of the remnant in the apical third [38,39]. According to a qualitative examination by a previous study [39], the XPF was the most efficient way to remove aggregated mineralized tissue remnants in the apical third. Additionally, the XPF is reported to preserve dentine. This finding disagrees with a previous study which concluded that XPF removed less Ca $(OH)_2$ in the apical third than in middle and coronal regions and could be related to the change in instrument shape to a spoon upon rotation in the canal and expansion of the middle part of the instrument by more than its tip [40].

At room temperature, the XPF is a straight instrument in the martensite phase, but when heated to body temperature, it transforms into the austenite phase and takes on a spoon-like shape. As the file is rotated in an up-down motion, this configuration results in expansion followed by contraction while touching the internal dentinal surface of the canal and agitating the irrigants. During the expansion and contraction of the file, in accordance with the root canal morphology, the file can reach and clear places which are normally hard to be adequately debrided [41]. Thus, an immature straight root canal can be sufficiently debrided with the aid of the XPF which could be considered as an adjunctive irrigation technique for the elimination of DAP.

According to a previous study, the XPF showed a greater root canal cleaning efficacy than the passive ultrasonic irrigation technique [42]. The root-filling material mean reduction (bacteria, AHTD, and organic tissues) obtained with XPF was equal to 63.84% [16,41,42]. Conventional needle irrigation and passive ultrasonic irrigation guaranteed a mean reduction of 44.82% [41] and 64.12% [16,17,42], respectively. Another study concluded [43], the best efficacy of removing of the calcium hydroxide from the apical part of the canal was obtained in the XPF group in both dimensions (96.32% and 91.35%). Additionally, similar results were achieved in the Brush-Finisher group (89.68% and 81.85%) without any significant difference.

In the present study, PEG400 when used as a vehicle showed the least amount of remnants apically in comparison to the rest of the groups. This finding is in agrees with previous study which concluded that using both of NaOCl and EDTA with sonic activation might improve the removal results of CH mixed with distilled water or propylene glycol [44].This findings do not coincide with those of a prior investigation that examined whether there are any residues left behind after Ca $(OH)_2$ has been removed that are mixed with propylene glycol, silicone oil, and chlorhexidine gluconate. Regardless of whichever vehicle used, remnants of the medication were observed in all the roots [45].

The major limitation of this study is the results obtained from *in vitro* conditions. Thus, future studies should be carried out to confirm the results in clinical condition.

4.1. Conclusion

Within the limitations of this study.

It can be concluded that the complete removal of DAP from the root canal wall using XPF is a difficult task regardless of the vehicles used. However, PEG 400 group reported the lowest remnant percentage, especially in the apical third.

4.2. Recommendation

Further studies on a new removal technique to enhance the removal of DAP mixed with different vehicles from immature root canals.

Ethics information

The Research Ethics Committee (REC) of the faculty of Dental Medicine for Giles Al-Azhar University (REC-CL-23-07). This study was done at Endodontic department, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt; Cairo, Egypt.

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Conflict of interest

There are no conflicts of interest.

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