Original Article

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Clove oil and supplementary irrigation techniques effectively removed parachloroaniline precipitate from root canal walls

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Objectives: To compare paracholoaniline precipitate solubility of xylene, GP solvent, and clove oil and evaluate the remaining precipitate on root canal walls after irrigation with the most effective solvent combined with different supplementary irrigation techniques.

Materials and Methods: Thirty-four human mandibular premolars with single root canals were used. Fourteen-mm decoronated roots were instrumented and irrigated with NaOCl and EDTA. Specimens were irrigated with NaOCl and CHX to create paracholoaniline precipitate. Fifteen roots were divided into 3 organic solvent groups: xylene, GP solvent and clove oil. Two roots were irrigated with distilled water. Each root was split in half and mounted on customized-remountable silicone jig. Root canal wall images were captured via light microscopy, then rinsed with organic solvent and distilled water. Root pieces were remounted and post-solvent images were captured. Orange-brown precipitation area was evaluated. Data was recorded as initial and remaining precipitation area. Paracholoaniline precipitate solubility means were compared using Welch Anova followed by Games-Howell for multiple comparisons. Next, fifteen roots were divided into 3 irrigation technique groups: needle syringe, passive ultrasonic technique, and XP finisher file. Two roots were irrigated with distilled water. The most effective solvent, clove oil, was used. Roots were split, mounted and captured at coronal, middle and apical portion. Percentages of remaining precipitate median were compared using Kruskal-Wallis and Freidman tests. The significance level was set at 0.05.

Results: Clove oil, xylene and GP solvent demonstrated 99.6, 8.43, and 3.57% paracholoaniline precipitate solubility, respectively. Mean percentages were significantly different ($p<0.05$). Clove oil was the most effective solvent. When irrigating with different techniques, percentages of remaining precipitate were not significantly different ($p>0.05$).

Conclusion: Clove oil was the most effective solvent in dissolving paracholoaniline precipitate. Xylene and GP solvents have minimal solubility. Rinsing with clove oil using needle syringe dissolved the paracholoaniline comparable with other techniques.

Keywords: chemical precipitation, chlorhexidine, clove oil, root canal irrigants, sodium hypochlorite

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Introduction

The eradication of microorganisms colonizing the root canal system is the main objective of mechanical instrumentation. However, root canal instrumentation alone cannot completely eliminate the intracanal bacterial load [1]. It is strongly recommended to use antibacterial irrigants, such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX), during mechanical instrumentation to enhance the antibacterial effect [2].

NaOCl is the most commonly used irrigant for root canal disinfection and typically used in concentrations of 0.5–6%. It is a strong base (pH>11). NaOCl is antimicrobial and dissolves

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organic tissue. However, high concentration NaOCl is toxic and is an irritant if accidentally extruded into the periapical tissues. CHX is used as a broad-spectrum antimicrobial agent. It has substantivity, lower toxicity, and is effective against persistent infection [3].

NaOCl is the main root canal irrigant during chemo-mechanical preparation. Using NaOCl with varying concentrations revealed that bacteria are not completely removed from the root canals [4, 5]. Irrigation with NaOCl followed by CHX results in greater bacterial reduction than NaOCl alone [6]. A final rinse with CHX significantly reduced bacterial loads in root canals that had been irrigated with NaOCl during canal preparation [7]. Moreover, CHX is more effective than NaOCl in eradicating persistent infection [8]. Therefore, in single-visit root canal treatment [9] and persistent infection [10], irrigation with CHX after irrigation with NaOCl was proposed as a disinfection guideline.

Although NaOCl and CHX are recommended to be used together in the same visit, it is critical for clinicians to be aware of their possible interactions. When NaOCl and CHX are mixed, a peach to brown-orange precipitate is produced. The color darkened and the precipitate thickened as the concentration increased [11-14]. Several studies used various methodologies to assess the composition of the precipitate. The presence of Parachloroaniline (PCA) was detected from time-of-flight ion mass spectrometry (TOF-SIMS) [11, 14, 15], Gas chromatography/Mass spectrometry [16, 17], and Proton nuclear magnetic resonance (^{1}H NMR) [18, 19]. PCA has a chemical formula of C_6H_6ClN. It dissolves in organic solvents and oil and has a water solubility of 2.6 g/L at 20°C [20].

Parachloroaniline is rapidly absorbed via inhalation, ingestion, and skin contact. The reactive metabolites of PCA covalently bind to haemoglobin and liver and kidney proteins. The PCA causes methaemoglobinemia, cyanosis and acts as a carcinogen with repeated exposure [20]. PCA precipitation affects root canal treatment procedures because it acts as a chemical smear layer, adheres to root canal walls, and occludes dentinal tubules [15, 21]. This compromises dentin permeability [22] and the sealing ability of root canal treatment procedures [23-25]. Moreover, PCA precipitation may be toxic to periapical tissues [11].

To prevent these problems, it is recommended that the NaOCl should be absorbed with paper points before a final rinse with CHX [9, 21]. Another option is to irrigate the canal with absolute alcohol, saline or distilled water as intermediate flushes [19, 26]. A previous study found that if these techniques did not completely remove the remaining NaOCl, the interaction between NaOCl and CHX continued [19, 21].

Currently, the guidelines for PCA precipitate removal have not been established. The WHO report in 2003 revealed that organic solvents or oil can dissolve PCA precipitate [20]. The organic solvents used in endodontic treatment comprise xylene, orange oil, and clove oil. Xylene and orange oil are gutta percha solvents in endodontic retreatment [27, 28]. Clove oil is a therapeutic agents and solvents in dental materials such as root canal cement and temporary filling [29, 30]. However, the high viscosity of these solvents impedes their flow to the apical region of root canal system [31]. To facilitate the area of fluid movement, supplementary irrigation may be suggested [32]. The commonly used effective supplementary irrigation methods are passive ultrasonic irrigation (PUI) [33] and XP finisher (XPF) rotary file [34].

Materials and Methods

The study was approved by the Institutional Review Board of the Faculty of Dentistry and Faculty of Pharmacy, Mahidol University (MU-DT/PY-IRB 2022/011.0402).

Tooth selection and preparation

Thirty-four extracted human mandibular premolar teeth were collected from a public hospital and private dental practice. The teeth were collected from patients 20 – 50 years old. Teeth without root caries, cracks, root fractures, or previous root canal treatment were selected and stored in 0.1% thymol solution (M Dent, Bangkok, Thailand) until used. Calculus, bone, and residual soft tissue were removed from the teeth using an ultrasonic cleaner (Newtron® P5XS, Acteon, Merignac cedex, France). Moreover, radiographs were taken in the mesiodistal and buccolingual directions to include teeth with a single straight root canal and curvature less than 15°, the Schneider method was used to determine the degree of curvature [35].

The teeth were decoronated with a diamond disc (EDENTA AG, AU/SG, Switzerland), to obtain a 14-mm root length. The working length (WL) was confirmed with no. 10 K-files (Dentsply Maillefer, Ballaigues, Switzerland). The WL was set 1 mm short of the apical foramen. The teeth with apical foramens no larger than size 25 K-file were included.

The root canals were instrumented with Wave One Gold files (Dentsply Maillefer, Ballaigues, Switzerland) to a large file (45/05) with a reciprocating motion (clockwise 150 and counter-clockwise 30) using a wireless electric motor (E-connect S, Eighteeth, Jiangsu Province, China). Recapitulation was performed using a 15 K-file to maintain apical patency. During instrumentation, the canals were irrigated with 1 mL 2.5% NaOCl (M Dent, Bangkok, Thailand) between each file and a final irrigation with 3 mL 17% EDTA (M Dent, Bangkok, Thailand) for 1 minute, using a 27-gauge irrigation needle (Nipro Corporation Limited, Ayutthaya, Thailand), 1 mm short of the WL using a short up and down motion. Longitudinal grooves were made along the buccal and lingual root sides with a diamond disc, without perforating the root canal.

PCA model preparation

PCA precipitation was created in the root canals by rinsing with 2 mL 2.5% NaOCl followed by 2 mL 2% CHX (CU, Bangkok, Thailand), using a 27-gauge irrigation needle inserted 1 mm short of the working length with a short up and down motion. The final rinse was 5 mL distilled water. The rinsing time was 1 minute per solution.

Part 1: PCA solubility test of xylene, GP solvent, and clove oil

Seventeen roots were split in half with a chisel and mallet. The specimens were mounted on a silicone jig. A 4 x 3 mm area 1 mm from the top edge of the specimen was examined and captured using a light microscope (CX31RTSF, Olympus Corporation, Tokyo, Japan) at 40X magnification before PCA dissolution.

The orange-brown precipitate area was calculated by Image-Pro Plus 9 (Media cybernetics, Maryland, USA) and recorded as the Initial precipitation area (P_1) (mm^2). The specimens were divided randomly into 3 groups according to the 3 organic solvents used.

Group 1: Xylene (n=5) Specimens were directly rinsed with 1 mL xylene (M Dent, Bangkok, Thailand) using a glass syringe for 1 minute, then rinsed with 5 mL distilled water to eliminate the solvent and stop the reaction.

Group 2: GP-Solvent (n=5) Specimens were directly rinsed with 1 mL GP-solvent (Nippon Shika

Yakuhin, Shimonoseki, Japan) using a glass syringe for 1 minute, then rinsed with 5 mL distilled water

Group 3: Clove oil (n=5) Specimens were directly rinsed with 1 mL clove oil (M Dent, Bangkok, Thailand) in a glass syringe for 1 minute, then rinsed with 5 mL distilled water.

Negative control (n=2) Directly rinsed with 6 mL Distilled water

After irrigation, the specimens were blotted dry and remounted on a silicone jig in the same position. The specimens were re-evaluated and captured using a Light Microscope at 40X magnification, and the remaining orange-brown precipitate area was calculated by Image-Pro Plus 9. The area of precipitation was recorded as the Remaining precipitation area (P_2) (mm^2).

The percentage of PCA precipitation solubility was calculated using the following formula

$$= \frac{\text{Initial precipitation area } (P_1) - \text{Remaining precipitation area } (P_2)}{\text{Initial precipitation area } (P_1)} \times 100$$

Part 2: PCA solubility evaluation of the most effective solvent (MES) from Part 1 combined with supplementary irrigation techniques

Fifteen specimens were divided into 3 groups according to the 3 irrigation techniques.

Group 1: Needle syringe (n=5) Irrigated with 1 mL MES for 1 minute with a 27-gauge needle, 1 mm short of the working length with a short up and down motion. The final flush was 5 mL distilled water.

Group 2: PUI (n=5) Irrigated with 1 mL MES for 1 minute using an IrriSafe file K 25 (Acteon, Merignac cedex, France) with P5 Newtron XS, power setting 7. The file was inserted 1 mm short of the working length. The final flush was 5 mL distilled water.

Group 3: XPF (n=5) Irrigated with 1 mL MES for 1 minute with XP finisher rotary files (FKG Dentaire SA, La Chaux de Fonds, Switzerland) in water bath 37˚C. The file was used at the WL with 1000 rpm and 1 Ncm of torque with slow in-and-out 7-8 mm motion. 7-8 mm.

Negative control (n=2) Irrigated with 6 mL distilled water rinsing for 1 minute with a 27-gauge needle, 1 mm short of the working length with a short up and down motion.

Each specimen was split longitudinally using a chisel and mallet into 2 pieces. The specimens were evaluated and included at least one piece of specimen that had all root canal portions. The halves were equally divided to coronal, middle and apical portions and reference lines were marked at the outer border of the root. The specimens were examined using a Light microscope at 40X magnification and the images were captured with a camera and the area was calculated by Image Pro-Plus 9. The data were recorded as overall root canal area (μm^2) and remaining precipitation area (μm^2).

Percentage of remaining PCA precipitate was calculated using following formula

$$= \frac{\text{Remaining precipitation area } (\mu m^2)}{\text{Overall area } (\mu m^2)} \times 100$$

Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 23 (IBM SPSS Inc., Chicago, IL, USA). Part 1, the data was normally distributed using the Shapiro-wilk test, non-homogeneity of variance using the Levene's test. The mean percentage of PCA precipitate reduction was analysed using Welch Anova followed by Games-Howell for multiple comparison. In Part 2, the data were not normally distributed using the Shapiro-wilk test. The median percentage of the remaining PCA precipitate was analysed using the Kruskal-Wallis's test and Friedman's test. The significance level was set at 95%.

Results

The specimens were evaluated using a light microscope. An orange-brown precipitate in various sizes and thicknesses on the root canal walls was detected along the length of the root canal in each specimen. Distilled water did not dissolve the PCA precipitate (Figure 3e). In the organic solvent groups, xylene (Figure 3f) and GP solvent (Figure 3g), large areas of orange-brown precipitate were typically seen. Whereas, in the clove oil group (Figure 3h), the precipitate was almost completely removed.

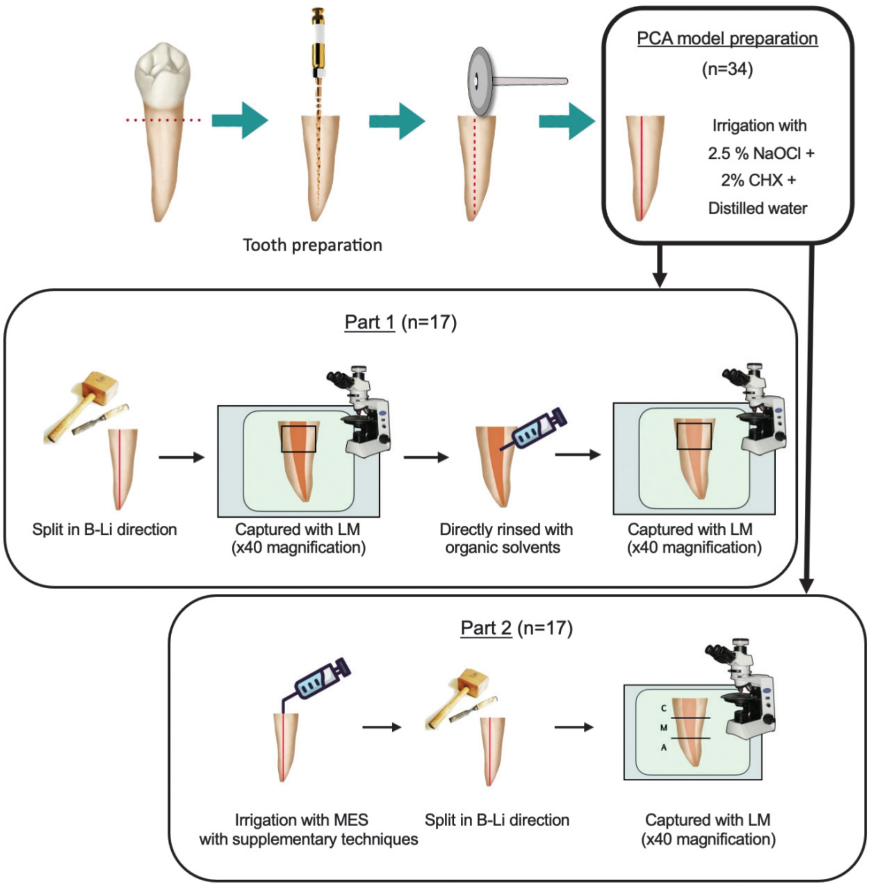

Figure 1 Experimental flow. PCA, Parachloroaniline; LM, Light microscope; MES, the most effective solvent.

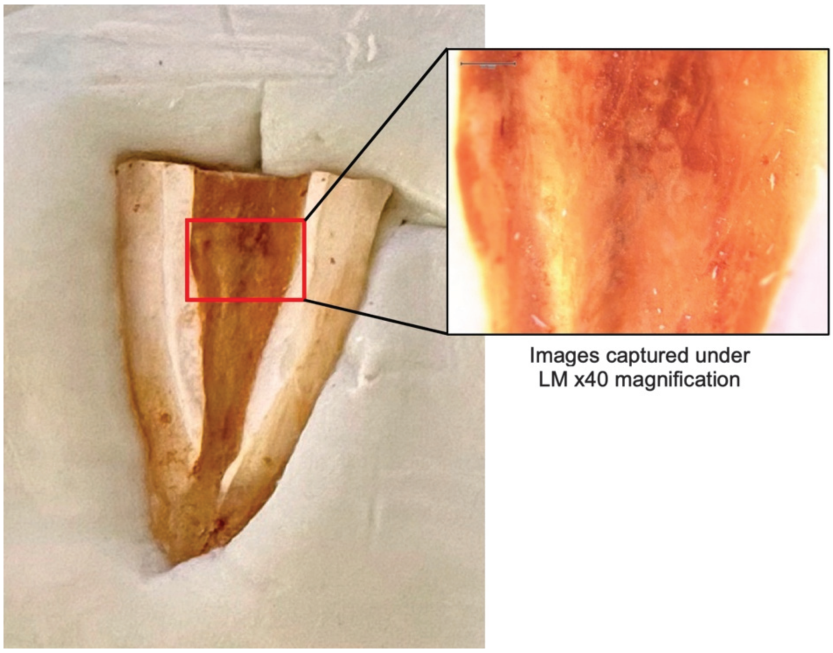

Figure 2 Specimen was mounted on a silicone jig. Red frame showed a position of specimen used for evaluation. Black frame showed an image captured under Light microscope x40 magnification.

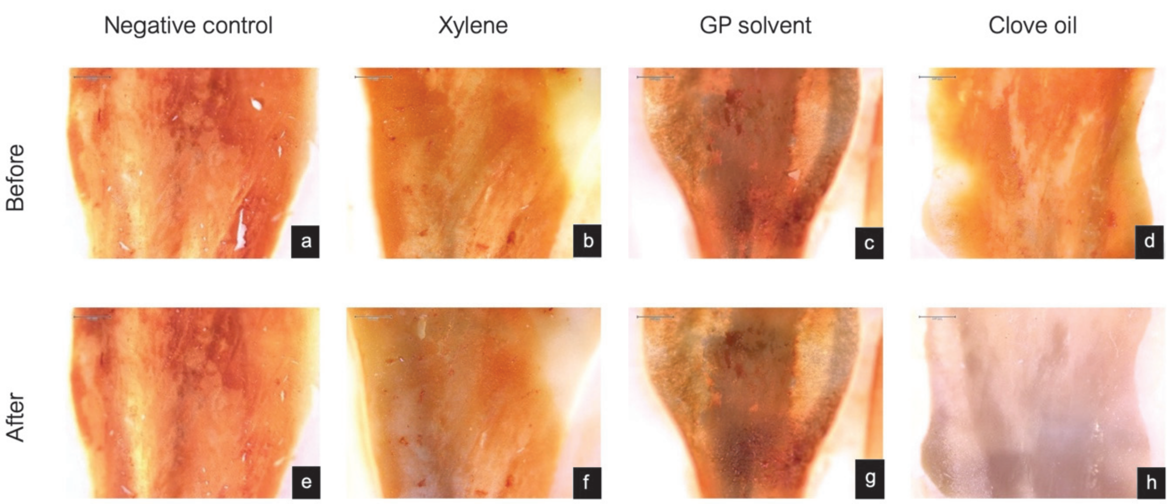

Figure 3 Light microscope images of the specimen showed before and after PCA solubility.

The mean and standard deviations of percentage of PCA dissolution were highest in the Clove oil group (99.66 % ± 0.28) followed by Xylene (8.43 % ± 2.88) and GP solvent groups (3.57 % ± 1.99). There were significant differences ($p < 0.05$) among the groups (Figure 4).

When irrigated clove oil with the different supplementary irrigation techniques, the median percentage remaining PCA precipitate at each root canal portion was not significantly different ($p > 0.05$). Comparing every portion within the syringe, PUI and XPF groups, the median percentage of remaining PCA precipitate was not significantly different in the coronal, middle or apical portions ($p > 0.05$) (Table 1). Using Clove oil with any of the irrigation techniques almost completely removed the PCA precipitate.

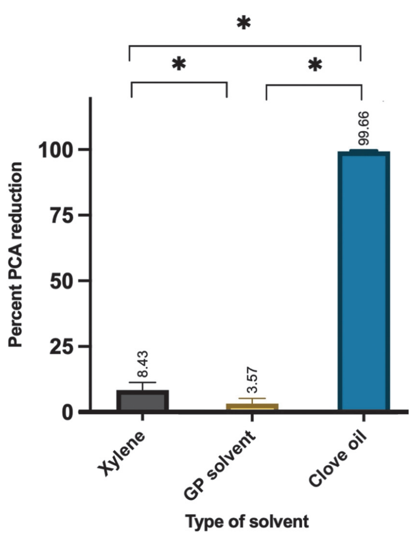

Figure 4 The percentage mean and standard deviations of PCA dissolution of 3 organic solvents.
* Indicated statistically significant difference ($p < 0.05$)

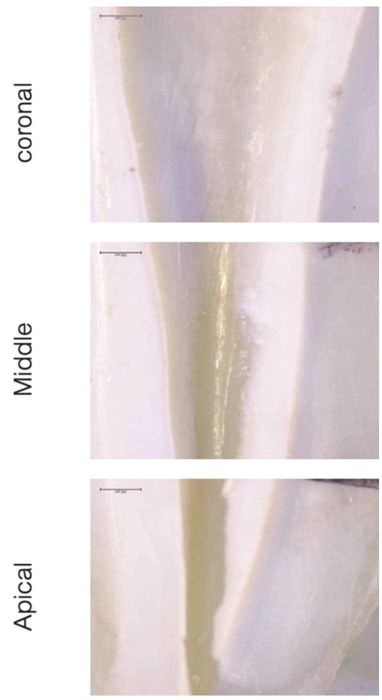

Figure 5 Irrigation using a needle syringe with clove oil almost completely dissolved the precipitate in the coronal, middle, and apical portions.

Table 1 Percentage of remaining PCA precipitate. There were no significant differences among the groups and root canal portions *($p>0.05$)*

Group	Percentage of remaining PCA precipitate						P-value
	Coronal		*Middle*		*Apical*		
	Median	(P_{25}, P_{75})	Median	(P_{25}, P_{75})	Median	(P_{25}, P_{75})	
Syringe	0.008	(0.002, 0.025)	0.004	(0.002, 0.015)	0.005	(0.003, 0.016)	0.549
PUI	0.005	(0.002, 0.028)	0.000	(0.000, 0.004)	0.004	(0.000, 0.056)	0.056
XPF	0.006	(0.002, 0.008)	0.002	(0.000, 0.034)	0.000	(0.000, 0.011)	0.143
P-value	0.811		0.143		0.208		

PUI : Passive ultrasonic irrigation, XPF : XP finisher rotary file

Discussion

The present study compared the PCA precipitate solubility of xylene, GP solvent, and clove oil and evaluated the remaining PCA precipitate on the root canal walls after irrigation with the most effective solvent combined with different supplementary irrigation techniques. After irrigating with NaOCl and CHX, an orange-brown precipitate formed almost instantaneously, due to an acid-base reaction. The precipitate adhered along the root canal walls [19, 32].

PCA has low water solubility and dissolves in organic solvents and oil (20). The results in this study revealed that organic solvents, xylene and GP solvent, slightly dissolved PCA and clove oil dissolved the precipitate very well. In contrast, Distilled water did not dissolve the precipitate.

Clove oil was highly effective (99.66 % ± 0.28) in dissolving the PCA precipitate from the root canal walls. When rinsing the canals with clove oil, the precipitate dissolved almost completely and there was very little precipitate left on the root canal walls. In contrast, xylene and GP solvents were slightly effective and distilled water had no effect on the PCA precipitate.

The principle of solubility is based on the Hansen solubility parameters. Two substances can dissolve when their Total Hildebrand parameter (∂_t) is similar or equal [36].

PCA is an aromatic hydrocarbon whose chemical formula is C_6H_6ClN. The ∂_t of PCA is 23.6 $MPa^{1/2}$. The organic solvents used in this study were xylene and GP solvent, and clove oil whose main constituent is xylene, D-limonene and eugenol, respectively. The ∂_t of xylene, D-limonene and eugenol is 18.04, 17.82 and 24.21 $MPa^{1/2}$, respectively [36]. The data reveal that the ∂_t of PCA and eugenol are very similar, however xylene and D-limonene have large differences. This explains why clove oil dissolved PCA very well.

Oils have higher viscosity than root canal irrigants, such as NaOCl. At room temperature, the viscosity of NaOCl is 1.073 centipoise (cP) [37], while that of clove oil is 6.6 cP [31]. Thus, clove oil is 6.6-fold more viscous than NaOCl. Therefore, the high viscosity of clove oil may not allow it to flow to the apical portion of the root canal. In this study, single root canal premolar teeth with straight and large root canals were chosen. Therefore, the solvent was able to flow to the apical root portion. The Part 2 results revealed that clove oil and the needle syringe technique dissolved the PCA precipitate along the length of the root canal walls. There was no significant difference when using clove oil combined with the other supplementary irrigation techniques, PUI and XP finisher file. Therefore, clove oil will dissolve PCA precipitate from the coronal, middle and apical portions using any irrigation technique. However, clinically, canals with complex anatomy, e.g., fins, isthmus, accessory and curved canals, may need supplementary irrigation techniques to enhance the accessibility of clove oil and improve PCA precipitate dissolution. This issue requires further investigation.

A light microscope was used for imaging in this study. The advantage of using a light microscope is that the color of the precipitate adhered to the root canal walls can be captured and calculated. However, its limitation is that it cannot determine the precipitate's thickness and the image can be captured only in one plane of the root canal wall. Due to the limitations of the light microscope, the flattest root canal walls were examined. In Part 1, the specimens were chosen from the coronal portion of the root canal walls, and the flattest and largest root canal area with the most initial precipitate was evaluated. Therefore, the decrease in solubility could be precisely calculated. In Part 2, the specimens were evaluated and included the best piece with a flat surface that can used to analyze the entire root canal wall.

Clove oil has been used in endodontics for many years. It has antimicrobial, anti-inflammatory, and solvent properties. It's generally combined with zinc oxide to form an amorphous chelate compound, which is a root canal sealer and temporary dressing [29]. Clove oil is increasingly recognized as a safer and effective alternative for dissolving precipitates like PCA. Incorporating these applications would emphasize the clinical relevance and versatility of clove oil in modern endodontic practice.

This is the first study that used clove oil to dissolve PCA precipitate. Furthermore, there is no report on using clove oil to irrigate the root canal. The main constituent of clove oil is eugenol [29]. There may be residual eugenol in the root canal after the final rinse with clove oil that can inhibit the polymerization of resin-based materials [38], which need further study. Therefore, various dental agents containing eugenol may dissolve PCA precipitate should be investigated.

This study demonstrated that a final rinse with clove oil dissolved PCA precipitate. It will be useful as a single-visit root canal treatment [9] and persistent infection [10] irrigation protocol. The PCA precipitate remaining in the root canal can be dissolved.

Conclusion

According to this experiment design, clove oil was the most effective solvent in dissolving PCA precipitate on root canal walls. Xylene and GP solvent demonstrated minimal solubility. Rinsing with clove oil using a needle syringe dissolved PCA as well as by other supplementary techniques.

HIGHLIGHTS

- This is the first study in which organic solvents were used to dissolve PCA precipitate from root canal walls.
- Clove oil was the most effective solvent in dissolving PCA precipitate.
- Supplementary irrigation techniques did not improve PCA precipitate solubility in large and straight-root canals.

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