Original Article

Comparative evaluation of the effect of ultrasonic and rotary agitation of herbal irrigating solutions on smear layer: A SEM study

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Abstract

Context: Instrumentation and irrigation balance helps in effective removal of endodontic microbes housing inside the smear layer.

Aim: This study aimed to (1) evaluate whether activation of the irrigating solutions with two different systems during the final irrigation step can lead to smear layer formation in the middle and apical third of the root canal and (2) evaluate and compare the smear layer removal ability of the aqueous extracts of *Emblica officinalis* and *Morinda citrifolia*.

Materials and Methods: A total of 72 single-rooted teeth were prepared up to F4 ProTaper. The specimens were assigned into eight groups of nine teeth each, according to the final irrigant and activation techniques. Further, the teeth were evaluated under SEM for endodontic smear layer at the middle and apical third.

Statistical Analysis: Inferential statistics included Pearson's Chi-square. Level of significance was set at 0.05 at 95% confidence level.

Results: Ultrasonic activation system showed significant ($P = 0.000$) amount of smear layer compared to XP-Endo Finisher file. A significant difference ($P = 0.00$) in the smear layer removal was observed when 6% *M. citrifolia* was activated with XP-Endo Finisher file both in the middle and apical third.

Conclusion: Within the limitations of this *in vitro* study, it can be concluded that smear layer formation was noted with ultrasonic and XP-Endo Finisher file when saline was used as an irrigant. 6% *M. citrifolia* when activated with XP-Endo Finisher file showed best results among all other experimental groups.

Keywords: 12.5% *Emblica officinalis*; 6% *Morinda citrifolia*; ultrasonic activation; XP-Endo Finisher file

INTRODUCTION

Chemomechanical instrumentation is of utmost importance for the successful treatment outcome in nonsurgical endodontic therapy. During the cleaning and shaping process, organic and inorganic debris accumulate and adhere onto the radicular canal wall producing a smear layer. Hand and rotary instruments produce smear layer of varied thickness on the canal walls as a consequence of the dentine cutting action.[1]

Sodium hypochlorite (NaOCl), a deproteinizing agent, is the most popular irrigating solution in endodontics, and its alternate use with EDTA, a calcium-chelating agent, has been recommended for the efficient removal of the smear layer. Different devices for irrigation delivery have been proposed to increase the flow and

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distribution of irrigating solutions within the root canal system.[2]

Passive ultrasonic irrigation (PUI), first described by Saber *et al.*, uses a stainless-steel activated file, which is passive without cutting or contacting the canal wall. It has been shown to be more effective than syringe needle irrigation in removing pulpal tissue remnants and dentin debris, bacterial reduction, and smear layer removal.[3,4]

XP-Endo Finisher™ file is an ISO 25/.00 instrument produced using a special type of alloy, the NiTi MaxWire (FKG Dentaire, La Chaux-de Fonds, Switzerland). It is designed to be used with irrigants after initial root canal instrumentation with the aim of removing vital and/or necrotic tissues and dentinal debris accumulated during instrumentation and smear layer.[1]

India is famous for its rich heritage of traditional medicinal systems and herbal remedies and holistic medicine have gained increasing popularity among the public, over the past decade. Furthermore, there has been a renewed interest in natural supplements and medicaments during the recent pandemic resulting in a paradigm shift toward the usage of phytochemicals in the field of dentistry in general and endodontics in particular.[5]

Various herbal irrigants that are currently being worked upon are triphala, green tea, polyphenols, *Emblica officinalis*, neem, *Morinda citrifolia,* and propolis.[2,6] However, their efficacy in the removal of smear layer is not well known. Evaluation of the capability of alternative irrigants to remove the smear layer is the need of the hour, particularly as they are continually replacing the conventional irrigants.[7]

E. officinalis has various properties like smear layer removal, antibacterial, antioxidant, and biocompatibility due to presence of gallic acid and tannins present in the fruit, which precipitate the protein and render them resistant to attack by proteolytic enzymes.[8]

Morinda has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects.[9,10]

Certain studies observed the formation of a smear layer on dentinal walls, even when no mechanical instrumentation had been performed; this observation motivated the present study to evaluate whether PUI and XP-Endo Finisher used could lead to smear layer formation and to evaluate smear layer removal ability of herbal irrigants.

MATERIALS AND METHODS

Specimen selection

A total of 72 intact human extracted teeth with single root were collected from the department of oral and maxillofacial surgery and appropriate disinfection protocols were followed. Teeth with well-formed root and having length of 20 mm or more were included. Whereas, teeth with severe root curvatures, developmental root defects, or fractured roots were excluded.

Root canal procedure

The samples selected were washed with distilled water and were subjected to ultrasonic scaling and stored as per OSHA norms until use.

The teeth were decoronated to obtain a standardized length of 14 mm. The canal patency was evaluated using a #10 K file. Working length of each sample was determined using digital radiography.

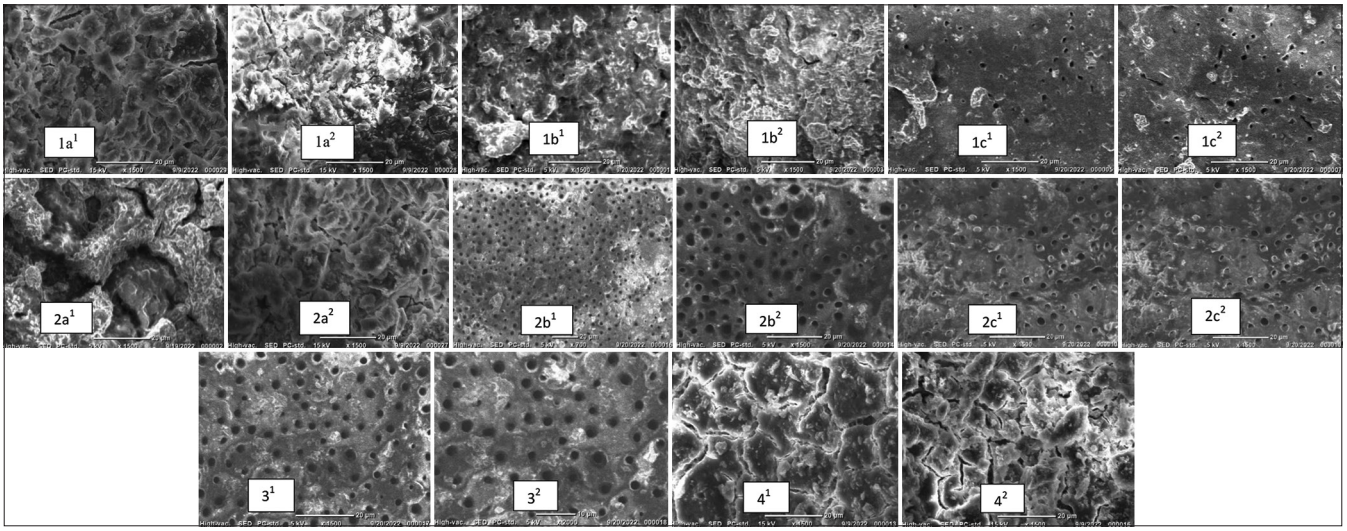
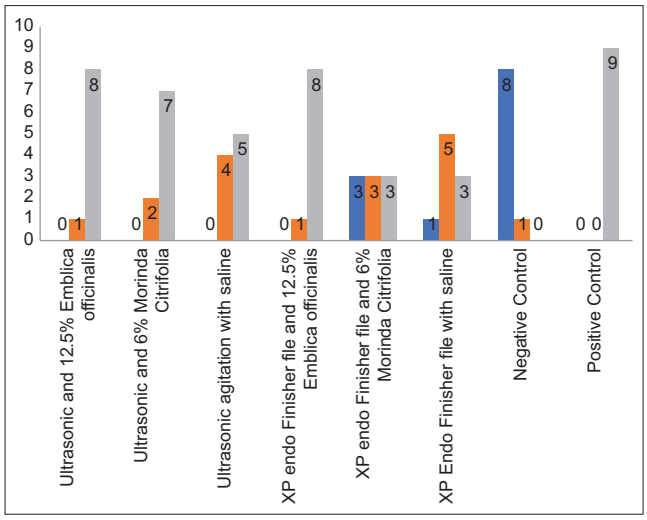
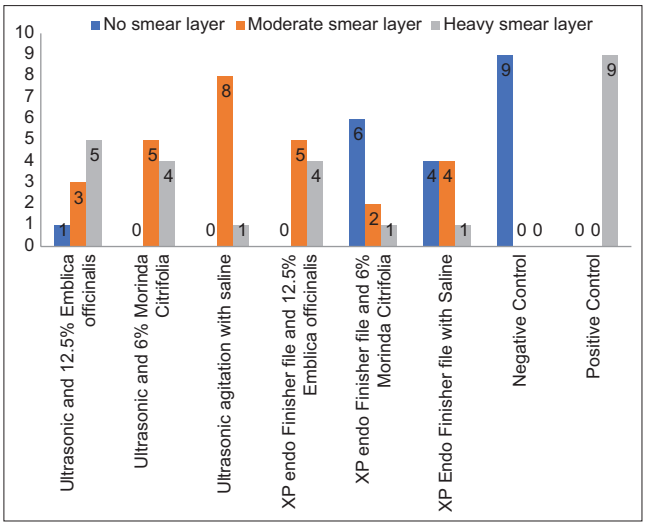


Figure 1: Scanning electron microscope images (×1500) of different groups (1-4) and subgroups (a-c). The superscript in the figure label depicts area of root canal imaged, respectively, as follows: 1 – middle third, 2 – apical third



Graph 1: Smear layer removal in middle one-third



Graph 2: Smear layer removal in apical one-third

Apices of each sample were filled with wax to prevent extrusion of the debris and irrigating solutions. All the canals were prepared up to F4 using ProTaper Universal NiTi rotary files (PU; Dentsply Maillefer, Ballaigues, Switzerland) by the same operator. The canals were irrigated with 5.25% NaOCl between each instrumentation and EDTA was used for lubrication and chelation. After completion of instrumentation, the canals were irrigated with saline.

The samples were then randomly divided into eight groups of nine teeth each, according to the final irrigant and activation technique.

- Group 1: Ultrasonic activation
  - Subgroup 1a: Ultrasonic agitation with 12.5% *E. officinalis*
  - Subgroup 1b: Ultrasonic agitation with 6% *M. citrifolia*
  - Subgroup 1c: Ultrasonic agitation with saline.
- Group 2: XP-Endo Finisher file
  - Subgroup 2a: XP-Endo Finisher file with 12.5% *E. officinalis*
  - Subgroup 2b: XP-Endo Finisher file with 6% *M. citrifolia*
  - Subgroup 2c: XP-Endo Finisher file with saline.
- Group 3: Negative control, ultrasonic agitation was done with 17% EDTA and 5.25% NaOCl
- Group 4: Positive control, rotary cleaning and shaping with saline and no agitation used.

Preparation of the irrigating solution

12.5% Emblica officinalis

Fruits of *E. officinalis* (Amla) were air-dried and pulverized into fine powder. The powders were subjected to cold maceration, followed by filtration with a sterile muslin cloth. Extract was dried using rotary evaporator. Distilled water extracts of each product were prepared by dissolving 12.5 g of dried crude extract in 100 ml of distilled water to obtain 12.5% aqueous extracts.[8]

6% Morinda citrifolia

The aqueous extract of *Morinda* was prepared by blending 6 g of pulp with 100 ml of distilled water.

All the samples were irrigated with 2 ml of the final irrigant of the respective group.

Longitudinal grooves were prepared along the buccal and lingual surfaces of the roots with a sectioning disk. The roots were then split using a chisel and a mallet. For SEM (JCM 6000 PLUS) analysis, the samples were dehydrated and coated with gold-palladium particles and a magnification of ×1500 was used, to evaluate the smear layer of the canal walls at middle and apical.

The presence or absence of smear layer on the surface of the root canal or in the dentinal tubules at the middle and apical portion of each canal was scored according to the following criteria:[11]

1. No smear layer – No smear layer on the surface of the root canals; all tubules were clean and open.
2. Moderate smear layer – No smear layer on the surface of the root canal, but the tubules contained debris
3. Heavy smear layer – Smear layer covered the root canal surface and tubules.

RESULTS

Pearson's Chi-square test revealed a significant difference ($P = 0.00$) among the subgroups in middle and apical third.

Removal of smear layer by herbal irrigants in middle third

Significant difference was seen ($P = 0.040$) among the subgroups (1a, 1b, 2a, 2b) with maximum smear layer

Table 1: Removal of smear layer by agitation systems and herbal irrigants

Group × smear layer cross tabulation							*P*
Count							
Middle/apical			Smear layer			Total	
			No smear layer	Moderate smear layer	Heavy smear layer		
Middle	Group	Ultrasonic and 12.5% *E. officinalis*	0	1	8	9	0.040
		Ultrasonic and 6% *M. citrifolia*	0	2	7	9	
		XP-Endo Finisher file and 12.5% *E. officinalis*	0	1	8	9	
		XP-Endo Finisher file and 6% *M. citrifolia*	3	3	3	9	
		Total	3	7	26	36	
Apical	Group	Ultrasonic and 12.5% *E. officinalis*	1	3	5	9	0.005
		Ultrasonic and 6% *M. citrifolia*	0	5	4	9	
		XP-Endo Finisher file and 12.5% *E. officinalis*	0	5	4	9	
		XP-Endo Finisher file and 6% *M. citrifolia*	6	2	1	9	
		Total	7	15	14	36	

E. officinalis: Emblica officinalis, M. citrifolia: Morinda citrifolia

Table 2: Smear layer removal by different agitation systems

Group × smear layer cross tabulation							*P*
Count							
Middle/apical			Smear layer			Total	
			No smear layer	Moderate smear layer	Heavy smear layer		
Middle	Group	Ultrasonic agitation with saline	0	4	5	9	0.000
		XP-Endo Finisher file with saline	1	5	3	9	
		Negative control	8	1	0	9	
		Positive control	0	0	9	9	
		Total	9	10	17	36	
Apical	Group	Ultrasonic agitation with saline	0	8	1	9	0.000
		XP-Endo Finisher file with saline	4	4	1	9	
		Negative control	9	0	0	9	
		Positive control	0	0	9	9	
		Total	13	12	11	36	

removal seen in the subgroup XP-Endo Finisher file and 6% *M. citrifolia* (2b) and least smear layer removal in the subgroup ultrasonic and 12.5% *E. officinalis* (1a) and XP-Endo Finisher file and 12.5% *E. officinalis* (2a) [Table 1].

Removal of smear layer by herbal irrigants in apical third

Significant difference was seen ($P = 0.005$) among the subgroups (1a, 1b, 2a, 2b) with maximum smear layer removal seen in the subgroup XP-Endo Finisher file and 6% *M. citrifolia* (2b) and least smear layer removal in the subgroup ultrasonic and 6% *M.* citrifolia (1b) and XP-Endo Finisher file and 12.5% *E. officinalis* (2a). Whereas, ultrasonic and 12.5% *E. officinalis* (1a) showed better results in apical third compared to the middle third [Table 1].

Significant difference was seen ($P = 0.000$) among agitation techniques where maximum presence of smear layer is seen in positive control (Group 4) and least in negative control (Group 3). Ultrasonic agitation (1c) produced more smear layer compared to XP-Endo Finisher file (2c) [Table 2].

DISCUSSION

Instrumentation and irrigation of the root canal system is the cardinal section of endodontic treatment, for eradication of root canal microbes. Irrigation impacts was not just the main canal but also other anatomical complexities, including recesses, isthmuses, and ramifications. Therefore, adjunctive activation approaches have been proposed to propel the irrigant to these difficult-to reach areas.

Whenever dentine is cut using hand or rotary instruments, the mineralized tissues are shattered to produce considerable quantities of debris consisting of very small particles of mineralized collagen matrix which is spread over the surface to form the smear layer.

The thickness of smear layer has been evaluated by various authors reporting that the particles ranged in size from <0.5 to 15 μm. They also reported demonstrated a thin layer of grinding debris estimated it to be 2–5 μm thick, extending a few micrometres into the dentinal tubules. Whereas, some studies by Pashley *et al.* (1988) reported the thickness approximately 0.05–0.1 μm.[19,20]

The viable microorganisms in the dentinal tubules may use the smear layer as a reservoir for sustained growth and replication. The presence of a smear layer may also inhibit the action and effectiveness of root canal irrigants

and intracanal medicaments. Removal of the smear layer will allow proper disinfection and better adaptation of obturating materials to the canal wall.

Various alternative irrigants especially of herbal origin have been proposed. One of the herbal solutions which was evaluated in the present study was *M. citrifolia*. *Morinda* contains the antibacterial compounds l-asperuloside acid and alizarin.

A study by Saghiri *et al*. confirmed antibacterial and smear layer removal properties of *Morinda* and suggested that *Morinda* can be used as a root canal irrigant.[12]

The ability of 6% *Morinda* in smear layer removal along with EDTA was studied by Murray *et al.*[9] and found it as effective as 6% NaOCl. The smear layer removal ability in the apical third was less compared to coronal and middle third due to reduced irrigant flow in the apical third. Hence, in the present study, herbal irrigants were activated with activation devices, to increase their effectiveness and flow.[13] Significant difference ($P = 0.00$) in the smear layer removal was observed when 6% *M. citrifolia* was activated with XP-Endo Finisher file both in the middle and apical third.

The major constituent of *E. officinalis* is gallic acid. The superior efficacy of smear layer removal could be a result of its low pH (2.8–4.5). This fruit contains two hydrolysable tannins Emblicanin A and B, which has antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid, and glucose.[5]

In a study conducted by Bhargava *et al.*,[7] *E. officinalis* showed potential to remove smear layer as effective as EDTA. In the present study, 12.5% *E. officinalis* with ultrasonic showed better results in the apical third than in the middle third [Graphs 1 and 2]. As the pH increases, the availability of calcium ions from hydroxyapatite for chelation decreases. At the same time, a greater dissociation of the acidic irrigant produces an increased attraction for calcium ions. 12.5% *E. officinalis* activated with XP-Endo Finisher file has shown an average percentage of smear layer removal, which is similar to 12.5% *E. officinalis* with ultrasonic [Figure 1].

However, herbal irrigants cannot be a substitute for NaOCl considering its excellent tissue dissolving and antimicrobial property, but can be used as an alternate irrigant to reduce antimicrobial resistance and in patients who are concerned about the bitter taste of NaOCl.

Frequently used irrigation technique is the needle irrigation. Newer side-vented tips offer much more safer irrigation than open-ended needles. Agitation and constant replenishing of the irrigant offers increased effectiveness of the solutions. Various newer agitation systems have been tried for effective removal of smear layer like EndoVac, EndoActivator, Vibringe, Ultrasonic, XP-Endo Finisher, and lasers like PIPS and SWEEPS.[14]

Ultrasonic agitation uses frequencies that range between 25 and 40 kHz. The efficacy in irrigation is due to two phenomena: "cavitation" and "acoustic streaming." The effect of cavitation is limited and minimal, only at the tip of the instrument being used, whereas acoustic streaming has a more significant effect.[15] Alternate positive and negative pressure bubbles are created by the ultrasound in the liquid that they contact, which become unstable and collapse leading to an implosion resembling that of vacuum decompression. The detergent effect of ultrasonics is due to the explosion and implosion that releases impact energy.[16] Earlier study by Bao *et al*. concluded that acoustic streaming is responsible for effective cleaning of root canal walls.[17]

XP-Endo Finisher with its unique property to change its shape under the body temperature and to expand the range of action up to 6 mm from its axis can help explain its good performance. As the instrument contracts and expands in the canal, the irrigant solution is significantly activated and air bubbles are displaced, allowing the irrigant to flow into difficult-to-reach areas.[18]

In the study conducted by Simezo *et al*., PUI and irrigation with reciprocating activation systems caused dental erosion. The activation systems and irrigant solutions used alternatively for promoting the cleaning of canal walls have shown to produce dental erosion. This erosion indicates alteration in dentin properties, such as microhardness, mineral content, roughness, and modulus of elasticity.[19]

In the present study, ultrasonic activation system showed significant ($P = 0.000$) amount of smear layer. It could be argued that the metallic tip itself could have caused dental erosion but XP-Endo finisher flexible NiTi file system also produced significant amount of smear layer with saline.[20]

This study is in accordance with the study conducted by Kanaan *et al*., where the irrigant activation performed during the final irrigation step was associated with smear layer formation. In fact, all of the tested systems seem to have promoted formation of the debris adhering to the canal walls. This explains why no study in the related literature till date has reported an effective way of completely removing the smear layer in the apical third. However, these irrigant activation modalities have a converse effect of both smear layer reduction and also production of smear layer, although to a lesser extent in case of latter.[21]

The limitation of the present study is its laboratorial nature using extracted teeth and the nonavailability of commercial herbal irrigants.

Future research should focus on methodologies mimicking oral conditions using Simian specimen and other *in vivo* studies to correlate the findings.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that smear layer formation was noted during the final irrigation step with ultrasonic and XP-Endo Finisher file when saline was used as an irrigant. Ultrasonic activation system produced more smear layer compared to XP-Endo Finisher file.

6% *M. citrifolia* when activated with XP-Endo Finisher file showed best results both in the middle and apical third among all other experimental groups. The smear layer removal ability of 6% *M. citrifolia* was better compared to 12.5% *E. officinalis*.

Herbal irrigants can be used as alternate irrigant solutions to reduce antimicrobial resistance and in patients who are concerned about the bitter taste of NaOCl.

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Conflicts of interest
There are no conflicts of interest.

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