



## **Comparative Evaluation of the Efficacy of Newer Irrigating Solutions in the Removal of Smear Layer Using SEM**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author SP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author PKP managed the literature searches. Author TBVGR analysed the study. Authors MVN and DGD drafted the final manuscript. Author TS performed the statistical analysis. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** To compare the effect of EDTA, Phytic acid and Peracetic acid on smear layer removal by effective conventional irrigation.

**Materials and Methodology:** A total of 50 extracted mandibular premolars were selected. The canals were instrumented by rotary system up to F2 ProTaper and irrigated with 3% NaOCL simultaneously, teeth were divided into 4 groups according to the final irrigants: 17% EDTA; 2.25% PERACITIC ACID; 1% Phytic Acid; and saline. The canals were irrigated with 25 gauge side vented needles for 5 minutes. Specimens were examined under scanning electron microscopy (SEM). Smear layer removal was evaluated at coronal, middle and apical thirds of the root canal. The data is analyzed using the Kruskal-Wallis and Mann-Whitney U tests.

**Results:** In this study, the control group that is normal saline ( $4.05 \pm 1.06$ ) showed very less amount of smear layer removal. The highest amount of smear layer removal was seen in the group

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with phytic acid followed by peracetic acid and sodium hypochlorite groups. The apical third showed significantly more smear layer than the coronal and middle thirds ( $p < 0.05$ ). No significant difference was found between the coronal and middle thirds in all experimental groups ( $p > 0.05$ ).

**Conclusion:** 1% phytic acid was the most effective in removing the smear layer at each level of the root canal followed by 2.5% peracetic and 17% EDTA.

*Keywords:* Root canal; smear layer; endodontic therapy; SEM.

## 1. INTRODUCTION

The primary goal of an endodontic therapy is to obtain optimal cleaning and shaping, ensure effective microbial control, and complete and effective obturation of the root canal space. It is strenuous to achieve a sterile root canal with mere mechanical preparation due to the presence of complex anatomy of the root canal system [1]. Irrigation is a vital step during an endodontic therapy because it aids in elimination of microorganisms, tissue dissolution, cleaning and helps in chelating [2]. Various studies have shown that instrumentation leaves a smear layer which is an amorphous, irregular layer covers the instrumented walls during cleaning and shaping [3]. Smear layer mainly contains organic and inorganic substances such as microbial debris, odontoblastic processes and necrotic debris [4]. Despite many arguments to maintain this smear layer, previous studies recommend removal of smear layer due to its impedious properties resulting from its constituents such as microbial debris. Smear layer removal is recommended because it improves the adaptation of the filling materials to the dentinal walls and allows the irrigating solutions and root canal medicaments to penetrate deeper into the dentinal tubules [5]. Ironically, many of the irrigating solutions now a days fail to eliminate the smear layer, especially from the apical third of the root canals [6]. So, in the present context, the focus is on finding an ideal root canal irrigant which has desirable properties such as effective disinfection with adequate lubrication and flushing action, antibacterial properties, ability to dissolve organic and inorganic tissues, non-toxic to surrounding tissues and without weakening the tooth structure [7]. It should retain its effectiveness with dental hard tissues and when it mixed with other irrigants, it should possess low surface tension. However, no one irrigant possess all these properties together. Currently, sodium hypochlorite (0.5%-6.5%) and EDTA (15%-17%) are the most commonly used irrigants. Sodium hypochlorite dissolves the organic tissue whereas EDTA is a chelating agent for inorganic divalent cations including  $Ca^{+2}$

ions forming  $Ca^{+}$  chelates. Regardless of its action, it has also been reported that application longer than one minute might cause deleterious effects and cause detrimental alterations in the root canal dentine [8,9].

Phytic acid (IP6, INOSITOL HEXAPHOSPHATE) is an organic acid which is a major storage form of phosphorus in plant seeds and bran which can be extracted in low costs. It contributes in a variety of cellular functions. Phytic acid contains multiple negative chains, making it an effective chelator of various cations such as calcium, magnesium and iron. Nassar et al, [10] found that phytic acid has the potential to be used as a possible alternative chelating agent for the removal of the smear layer due to its ability to chelate multivalent cations [11].

Peracetic acid (PAA) is one of the potent disinfectants and has been researched with the purpose of improving the cleaning and disinfection of root canal system [12-15]. Previously it has been used as a single endodontic irrigant in Europe [16]. It has broad spectrum of action against various bacteria, fungi, viruses and has sporicidal effects. The peracetic acid content seems to cause inorganic material dissolution while also forming complexes with calcium. In the previous study done by Lottanti et al. it has been proved that 2.25% PAA solution is equivalent with 17% EDTA at removing smear layer.

## 2. METHODOLOGY

### 2.1 Tooth Selection and Preparation

Teeth were stored in 0.1% thymol solution at  $5^{\circ}C$  for no more than 1 year. The crowns of all teeth were removed to standardize to a full root length of 13 mm as measured from the apex using a laboratory hand piece and a diamond-coated micro saw. To prevent calcium from being eluted from outer root surfaces, these were covered with nail varnish. A #10 K $\bar{}$ file (Mani Inc., Japan) was inserted beyond the apex in order to confirm patency. To establish the final length to which the canals can be instrumented, 1 mm was

subtracted from this length. The canals were gradually enlarged, and a glide path established with hand instruments to a size #15 K<sup>®</sup> file (Mani Inc., Japan). In the presence of 3% NaOCl (Vishal Dental Products, India), nickel–titanium universal rotary ProTaper was used to shape the canal up to F3 ProTaper (Dentsply Maillefer, Switzerland). Cleaning and shaping of all the samples are done by using ProTaper files (S1 to F2) along with irrigation with 3% NaOCl irrigating solution using 30-G side-vented needles between each file. Later, all the samples were divided into four groups according to the final irrigating solution.

The treatment groups were as follows

- Group A (control) n=5: 3% NaOCl for 5min- distilled water for 3 minutes
- Group B (n=15): 3% NaOCl for 5 min- 1% phytic acid for 3 minutes
- Group C (n=15): 3% NaOCl for 5 min- 2.5% Per acitic acid for 3minutes
- Group D (n=15): 3% NaOCl for 5 min- 17% EDTA

After the final irrigation, canals were irrigated with 2 ml of distilled water and dried with absorbent paper points. For sectioning of the tooth, two longitudinal grooves were prepared on the buccal and lingual surfaces using a diamond disc so that penetration to the root canal was avoided. Each root was further split into two longitudinal parts using a chisel and a mallet. Fifteen root halves were obtained for each group. The specimens were then placed in ascending concentrations of ethanol solutions.

All specimens were then dried overnight inside a closed glass vial and then sputter<sup>®</sup> coated with gold and observed under SEM. SEM photomicrographs were captured at a magnification of ×1000 in order to evaluate the smear layer at different levels of root canals. To grade the smear layer removal from root canal walls 3 calibrated examiners viewed the SEM photomicrographs, and analyzed independently and in a blind manner, using the 5<sup>®</sup>-point scoring system by Hülsmann, et al. All the results were tabulated.

- Score 1: No smear layer present and all dentinal tubules open.
- Score 2: Small amount of smear layer present and some dentinal tubules open.

- Score 3: only a few dentinal tubules open and homogenous smear layer covering the root canal wall.
- Score 4: No open dentinal tubules and complete root canal wall covered by homogenous smear layer.
- Score 5: Heavy, nonhomogeneous smear layer covering the complete root canal wall.

## 2.2 Data Presentation and Analysis

The smear score comparisons were done using Kruskal–Wallis analysis of variance followed by the Mann–Whitney U test. These values are presented as medians and ranges. Bonferroni’s correction for multiple testing was applied for all individual comparisons. The alpha type error was set at 0.05

## 3. RESULTS

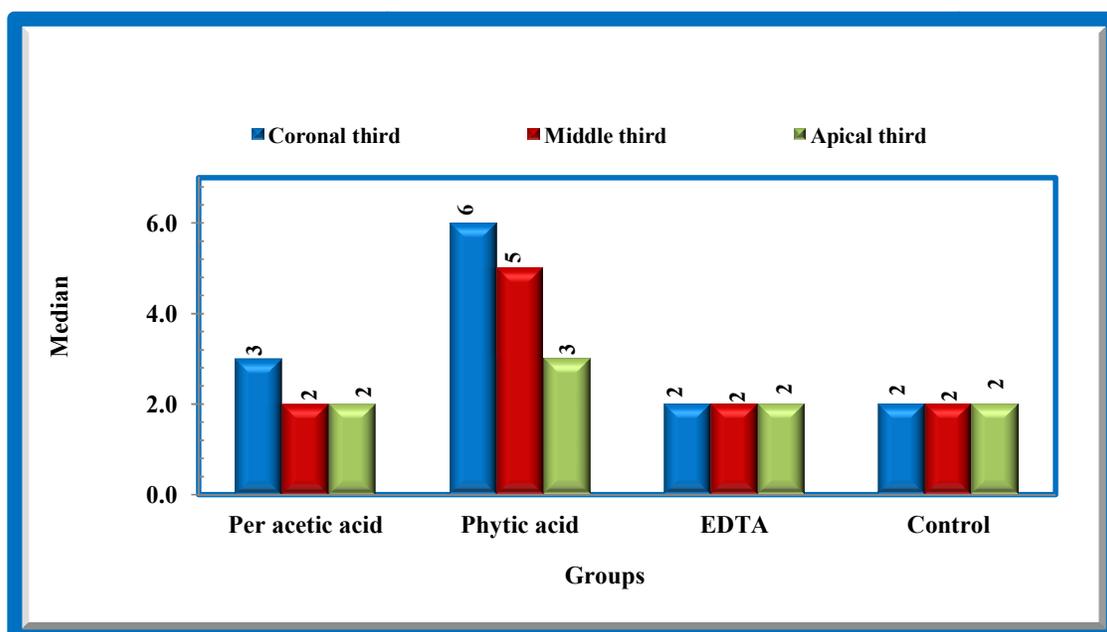
The statistical parameters: for each group mean, standard deviation along with median of smear layer removal scores were obtained as shown in Table 1. The mean for control (normal saline) at the apical third of the root canal section showed the lowest results while that of phytic acid at the coronal third was the highest.

Fig. 1 demonstrates the comparative percentage of smear layer removal at the coronal, middle, and apical thirds of the root canal by different irrigants.

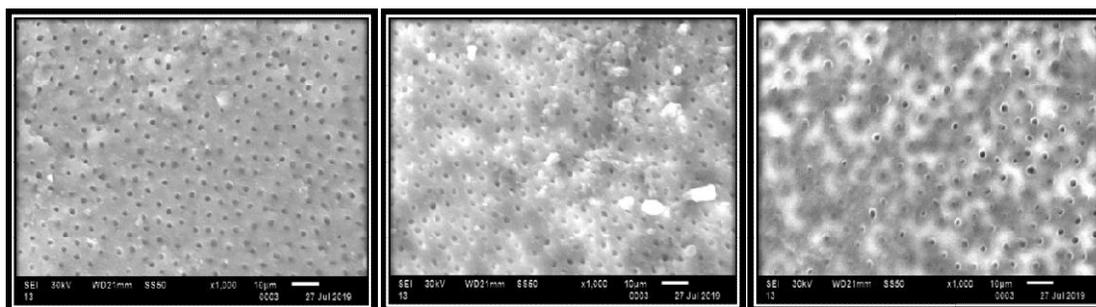
A highly significant difference was seen between the phytic acid, PAA, and EDTA groups in smear layer removal at the apical third of the root canal ( $P < 0.05$ ). There was no significant difference between the EDTA and the PAA groups in smear layer removal from the coronal and middle thirds. Both phytic acid and PAA along with sodium hypochlorite removed smear layer efficiently in the coronal third of the root canal walls. In the coronal third, PAA has shown smear layer removal equivalent to that removed by EDTA, but in the apical and middle third; its performance was better than saline. However, in the middle and apical third, phytic acid showed significantly better results when compared to PAA or EDTA [Fig. 2]. In EDTA and PAA treated root canal sections, there was the presence of moderate smear layer, and in some areas, [Figs. 3 and 4]. In control (saline) group, there was presence of highest amount of smear layer in all parts of the root canal walls [Fig. 5].

**Table 1. Score showing the smear layer removal of the groups at different levels of root canal represented in terms of mean±standard deviation (median)**

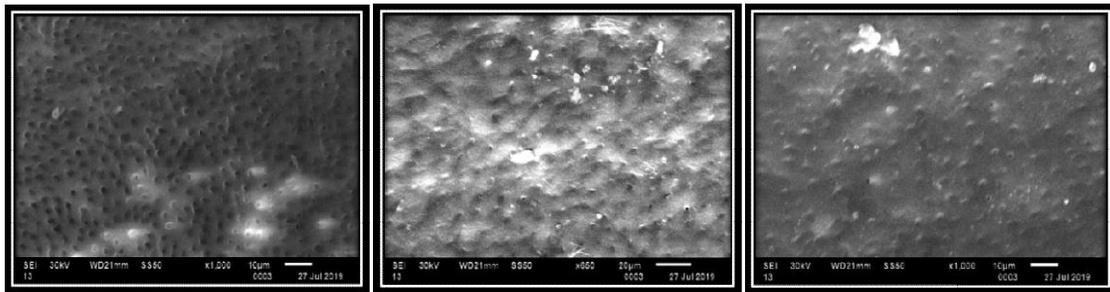
Parameters		Median	IQR	P-value
Per acetic acid	Coronal third	3.00	0.00	0.03*
	Middle third	2.00	1.00	
	Apical third	2.00	0.00	
Phytic acid	Coronal third	6.00	1.00	<0.01*
	Middle third	5.00	2.00	
	Apical third	3.00	1.00	
EDTA	Coronal third	2.00	0.00	0.99
	Middle third	2.00	0.00	
	Apical third	2.00	0.00	
Control	Coronal third	2.00	1.00	0.87
	Middle third	2.00	1.00	
	Apical third	2.00	1.00	



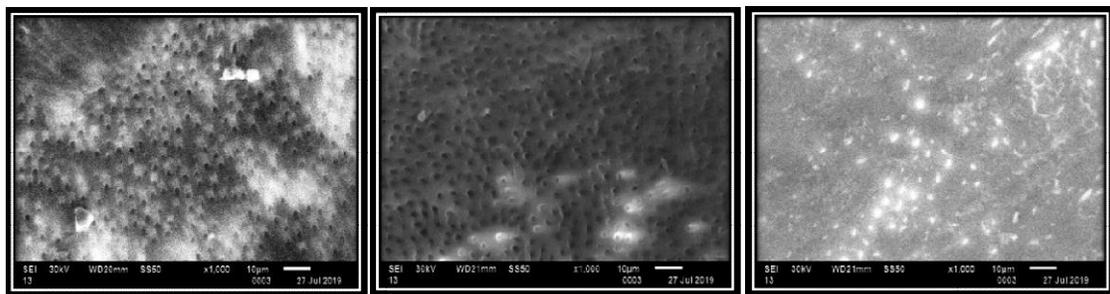
**Fig. 1. Comparison of the percentage of smear layer removal between different irrigant solutions at coronal, middle, and apical thirds of the root canals**



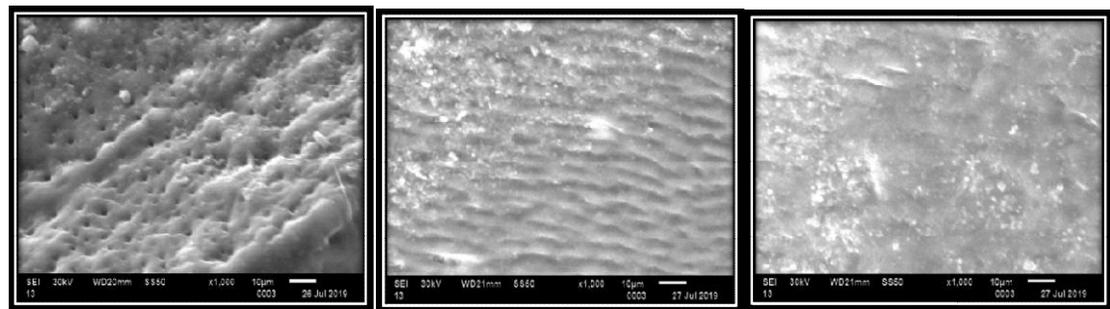
**Fig. 2. Scanning electron microscope - photomicrographs representative of 1%phytic acid, showing clean root canal at(a) coronal, (b) middle, and(c) apical thirds. Magnification: 1000 x**



**Fig. 3. Scanning electron microscope- photomicrographs representative of group 2.5% peracetic acid, showing clean root canals at(a) coronal,(b) middle, and (c) apical thirds. Magnification: 1000 x**



**Fig. 4. Scanning electron microscope- photomicrographs representative of group 17%EDTA showing moderate smear layer on root canal at(a) coronal,(b) middle, and(c) apical thirds. Magnification: 1000 x**



**Fig. 5. Scanning electron photomicrographs representative of group SALINE(CONTROL), showing excessive smear layer on root canals at(a) coronal, (b) middle, and(c) apical thirds. Magnification: 1000 x**

#### 4. DISCUSSION

The chemo mechanical debridement of root canals to clinically satisfactory levels is of paramount importance in the success of root canal therapy. Smear layer consists of necrotic tissue including leftovers of odontoblastic procedures, pulp tissue, and microorganisms and dentinal chips. Smear layer acts as a block and hinders the penetration of irrigants and root canal sealer within the dentinal tubules. Thus, choice of

irrigating solution should also be based on its smear layer removing ability. Therefore, it is a pre-requisite to have a clear knowledge on the underlying science and methods to achieve an adequate level of smear layer removal. In spite, NaOCl being the most commonly used irrigant in regular endodontic therapy, it is only known to dissolve the organic tissue, it does not have the ability to remove inorganic part of smear layer. Therefore, other irrigants were introduced.

In the present study, the smear layer removal ability of phytic acid showed statistically greater result than PAA and EDTA groups. Phytic acid is a newer plant based organic material which can be extracted with low cost from rice bran. The underlying chemistry of phytic acid has shown that it has strong binding affinity to many minerals such as calcium, iron and zinc. The 1% phytic acid solution has a Ph around 1.2 and this acidity, along with chelation ability, attributed to effective smear layer removal and Ca<sup>++</sup> extraction. The affinity of calcium with phytic acid is PH dependent leading to better chelating ability than conventional chelating agents. [17] In the present study, results are in accordance with a previous study done by Nassar et al in which phytic acid was found to be more effective in removing smear layer from NaOCl treated flat coronal dentin disc surfaces than EDTA. Nassar et al studied the chelating ability and biocompatibility of phytic acid and EDTA. He demonstrated that when compared with EDTA the effect of phytic acid led to cleaner root canals and widely opened dentinal tubules. According to his study, better biocompatibility to MC3T3-E1 odontoblast cells was seen with phytic acid which may contribute to better periapical bone healing when compared to EDTA [18]. In a previous study done by Kim et al., it was found that phytic acid had bactericidal effects which were much greater than those of other organic acids under the same experimental conditions.

Peracetic acid (PAA) is relatively cytotoxic [19]. Nevertheless, it is considered to be a healthy alternative to sodium hypochlorite for drinking water disinfection [20]. In the present study a 2.25% peracetic acid solution was used which is probably as caustic as a hypochlorite solution of the same concentration. The previous study done by Lottanti et al. [21]. showed that 2.25% PAA when used for 3 minutes after mechanical instrumentation led to a similar level of smear layer removal to that of 17% EDTA. The other studies done by Kühnfluck and Klammt et al. [22]. showed that PAA when used for around 60 seconds in contact with dentin at concentrations of 0.5%, 1% and 2.25% led to the dissolution of the smear layer as effectively as 17% EDTA. PAA is commercially available in the form of an aqueous solution, in which it is in equilibrium with hydrogen peroxide and acetic acid. The acetic acid component is probably responsible for the dissolution of the smear layer, as it forms complexes with calcium that are easily soluble in water [23].

In addition to the chelating ability of the irrigating solution in the process of smear layer removal, other requisites are the kind of irrigation technique used and amount of root canal preparation done. In this study, 30-gage side vented needles were used for effective conventional irrigation and apical preparation was done up to ProTaper F3.

In recent studies which employed a computational fluid dynamics model have shown that the tip design of the needle could probably affect the irrigant flow pattern, resulting apical pressure and its speed [24,25]. This concept showed that needles with side or beveled openings did not demonstrate advantages for irrigation of the apical region of the root in comparison to conventional needles with regular apical opening, which agree with the observations of the present study. Nevertheless, these modified needle tips have reduced the pressure generated at the apical foramen, which might decrease the risk of extrusion of the irrigant into the periapical tissues. Further studies focusing on the extrusion of irrigants should be performed to define the ideal irrigation needle tip design that incorporates efficacy and safety during endodontic irrigation.

## 5. CONCLUSION

Within the limitations of this study, 1% phytic acid was the most effective smear layer removal agent at each level of the root canal followed by 2.5% peracetic and 17% EDTA.

## CONSENT AND ETHICAL APPROVAL

With ethical consent from institutions ethical committee a total of 50 intact human single-rooted permanent mandibular premolar teeth with a single canal and fully developed apices, which were indicated for extraction due to orthodontic/periodontal reasons were selected for the study and preserved by the authors.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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