

Original Research**A comparative evaluation of effects of three chelating agents on smear layer of root canals of extracted human teeth-An In Vitro Study****Nikhil Mahanubhav¹, Tarun Ahuja², Zinnie Nanda³, Kranthikumar Reddy⁴, Juili Gawande⁵, Prasad Rane⁶**¹Post Graduate Student, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India²Professor, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India³Professor and Head, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India⁴Reader, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India⁵Post Graduate Student, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India⁶Reader, Dept of Conservative Dentistry & Endodontics, ACPM Dental College, Dhule, India

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ABSTRACT

Background: EDTA is used as agent of choice in preparation of canals, not many studies have been performed previously to find a substitute for EDTA. The aim of the study is to examine the chelating effects of EDTA and two additional agents viz. Citric acid and Deferoxamine.**Objective:** To compare the effect of three chelating agents on root canals of extracted sound human teeth.**Method:** 30 extracted non- carious single rooted teeth will be included in the study. Rotary instrumentation was performed with rotary files in a crown down fashion to a standardized master apical file #30. Teeth will be irrigated with 5 ml diiodized water and divided into 3 groups, 10 specimens in each. Each group will be treated separately with 1 ml of chelating agent as follows.

Group 1 – treated with 1 ml of 17 % EDTA for 5 min.

Group 2 – treated with 1 ml of 10 % citric acid for 5 min.

Group 3 – treated with 1 ml of 10 % deferoxamine for 5 min.

The teeth will be sectioned longitudinally. The two halves will be separated by a chisel to avoid touching the pulp chamber. The teeth will be mounted and put in a vacuum chamber for 2 weeks to dehydrate the sections. The middle third of root canals will be scanned at x 1000 using a JEOL, JSM-840 A under SEM.

Result: All the values will be recorded and subjected to statistical analysis.**INTRODUCTION**

Predictable successful endodontic therapy requires accurate diagnosis, proper biomechanical preparation and 3 dimensional obturation creating fluid tight hermetic seal. [1] However this seal is impaired by presence of micro crystalline debris known as smear layer formed due to instrumentation.

The newer generations of Nickel-titanium files are able to prepare only a large portion of the complex root canal architecture Thus a thorough irrigation protocol is

needed to disinfect and debride the deficient 35 % of the unreamed canal surface.[2] This primary motive of irrigation during debridement is generally overlooked.

A thorough debridement of canal is being recommended.[3] Various methods like chemical, ultrasonics and Lasers techniques have also been suggested to remove these tenacious smear layer. None of them have proved to be more operative and accepted universally. [4]

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Based on the reviews of literature of current endodontic therapy and irrigating intervention, the irrigants have potential to dissolve inactive endotoxins and are also known to impede or dissolve the smear layer. [5]

Various materials and techniques have been reported with wide variations in their efficacy regarding removal of the intra canal smear layer. [6, 7] The most widely used chemical for chelation is EDTA in different formulation. It has a claw like molecular structure which binds and seize ions. However its efficacy in narrow canal is questionable thus long operating contact time or excessive percentage usage can achieve optimal result [8] but this may severely damage the dentin structure and the surrounding area

Chelating agents such as citric acid and deferoxamine are used for root canal irrigation and Fe, Al ion binding respectively. The review of literature shows that not many studies are performed using citric acid and iron chelating agent deferoxamine against the smear layer removal. The aim of this in vitro study was to compare the efficacy of these chelating agent in removal of smear layer of root dentin under scanning electron microscope.

MATERIAL & METHODOLOGY

Ethical clearance was taken before starting the study. 30 extracted human anterior teeth with straight root and type I canal anatomy were selected. All the teeth were radiographed to verify the presence of single canal and mature apex. The exclusion criteria were previous endodontic treatment, calcification, open apex, internal or external resorption, root fracture, and any severe root canal curvatures.

The teeth were decoronated using a diamond disk (D&Z Damstadt ,Germany) to standardize the root length and the samples were divided into 3 groups (n = 10) according to the type of chelating agent used during

instrumentation. The working length was established by deducting 1 mm from the length recorded when tips of #10 or #15 k files (Mani, Tochigi Ken, Japan) were visible at the apical foramen (observed under magnifying loupes) . Rotary instrumentation was performed with Hero 0.04 taper rotary files (Micromega, Besancon france) in a crown down fashion to a standardized master apical file #30.

All teeth were irrigated with 5 ml diiodized water. Each group was treated separately with 1 ml of the chelating agent as follows:

- Group 1 was treated with 1 ml of 17% EDTA (Merck, Darmstadt, Germany) for 5 min
- Group 2 was treated with 1 ml of 10 % citric acid (Manipal pharmacy) for 5 min
- Group 3 was treated with 1 ml of 10 % deferoxamine (Manipal pharmacy) for 5 min

All teeth were then irrigated with 5 ml of diiodized water. The teeth were scored longitudinally using a high-speed fissure bur. A chisel was used to separate the two halves to avoid touching the pulp chamber and root canal space with the bur. The teeth were mounted and placed in a vacuum chamber for 2 weeks to dehydrate the sections. The sections were coated with palladium gold, using a RE RMC-EIKO Corp. sputter coat machine, in preparation for the scanning electron microscopic study. [10, 11] The middle third of the root canals were then scanned at x 1000 using a JEOL, JSM840A scanning electron microscope (SEM)

Smear layer were evaluated using following criteria:

Scores	Criteria
0	No smear layer and smear plug; no smear layer on

	the surface of the root canals. All dentinal tubules were cleaned and opened
1	No smear layer but mild smear plug; no smear layer on the surface of the root canals, small amount of smear plug in some dentinal tubules
2	No smear layer but moderate smear plug; No smear layer on the surface of the root canals. Most of the dentinal tubules had smear plug
3	Moderate smear layer; moderate smear layer covered the surface of the root canals; only few dentinal tubules were opened
4	Heavy smear layer; complete root canal wall covered by a homogenous or heavy non-homogenous smear layer, no opening of the dentinal tubules

Based upon the criteria used by torabinejad et al (9), the samples were given scores. According to smear layer evaluation criteria, score 0 was for complete cleaning of root wall and score 4 indicated heavy smear layer on the root canal walls.

STATISTICAL ANALYSIS

For analysis of smear layer removal, chi- square test and one way ANOVA test was used. Pair wise comparison of smear layer was done using Post Hoc-Tukey test. A significance level of 5% was adopted.

Table 2: Evaluation of smear layer removal in three groups

Groups	Score 0	Score 1	Score 2	Score 3	Score 4	Chi-square value	p value
EDTA	1	5	4	0	0	32.267	0.001*
Citric Acid	0	4	6	0	0		
Desferoxamine	0	0	0	3	7		

Chi-square test; * indicates significant at $p < 0.05$

Table 3: Score of smear layer in each solution

Groups	N	Mean Score	Std. Deviation	F value	p value
EDTA	10	1.30	0.675	53.686	0.001*
Citric Acid	10	1.60	0.516		
Deferoxamine	10	3.70	0.483		

One way ANOVA test; * indicates significant at $p < 0.05$

Table 4: Pairwise comparison of smear layer

Groups	Mean Difference	p value
EDTA vs Citric Acid	0.300	0.470
EDTA vs Deferoxamine	2.400	0.001*

Citric Acid vs Deferoxamine	2.100	0.001*
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Post hoc tukey test; * indicates significant at $p < 0.05$

RESULTS

According to the present study, the highest smear layer removal was observed with EDTA (1.3) followed by Citric acid (1.6) and deferoxamine (3.7)

Table 1 presents the scores based on criteria standardized for smear layer removal. Chi square and Anova test revealed EDTA, Citric acid and deferoxamine were significantly different from each other (table 2 & 3 resp) SEM analysis showed comparitibility of results in group I (EDTA), group II (citric acid) and group III (deferoxamine). The highest smear layer removal was observed with group I (EDTA)

Table 4 presents the distribution of mean and standard deviation of scores of smear layer removal of three groups in middle third of root canal

Post hoc tukey test was performed for intergroup comparisons, there was statistically significant difference within groups.

DISCUSSION

The smear layer has been described as one that is formed during biomechanical preparation, which plays a key role in endodontic success. Smear layer includes not only dentin but also necrotic and viable tissue along with remnants of odontoblastic processes, pulp tissue and bacteria. (10) It plays an important role in the lateral sealing of the root canal, as it acts as an intermediate physical barrier that may interfere with adhesion and penetration of the root canal sealer into the dentinal tubules. [11, 12] Pashley et al [13] had described the smear layer as a porous structure which is permeable to even large molecules like albumin. Mader et al [14] had stated that the smear layer is a non-homogenous and

weakly adherent structure which may slowly disintegrate and dissolve around leaky filling margins, thus creating voids between root canal walls and filling material / sealer.

The samples in the study were prepared with a crown-down technique using rotary nickel titanium instruments in rotational motion with torque and speed guided as per manufacturer's instructions. This technique was preferred because it is an effective method in preparing root canals with rotary instruments and the use of the rotary files creates a significant amount of smear layer. The apical portion of each canal was enlarged to a size 30 file to allow adequate cleaning and penetration of the solution to the apical third of each root canal. . In this study, the apical part of canal preparation was performed up to ISO size no. 30. This is in accordance with several other studies that have provided a strong consensus that larger apical preparation produces a greater reduction in remaining bacteria and dentin debris as compared with smaller preparation (15, 16)

The results of the study show citric acid was almost as good as EDTA. This can be attributed to the fact that since citric acid is highly acidic, it has a better demineralizing effect within a shorter period of time. Moreover, Vasiliadis et al (17) and Paque et al (18) reported that dentin in the apical third of the root canal is sclerosed. Hence, EDTA may not have such a pronounced action on sclerosed dentin also EDTA requires an application time of not less than 15 minutes for optimal results. Studies have reported that EDTA when used for more than 1 minute causes erosion of dentinal tubules, thus reducing the dentin microhardness and consequently causing root fragility (19, 20)

Jenkins et al [21] reported that citric acid is nature's chelating agent for dissolving the roots of deciduous teeth. It is possible, therefore, that the body has a built-in defense mechanism for neutralizing citric acid in dentin. Zehnder et al. [22] found that a 10% citric acid solution significantly removes more calcium than a 15.5% EDTA solution. In this study a nonconventional solution deferoxamine was used. it is a fe chelatng agent, not many studies and literature is available on deferoxamine in dentistry. Using it as an intracanal smear layer removal solution was probably a novel approach.

Having no negative control group i.e. group without treatment with chelating agents can be a limitation of the study. Also the sample size of the investigation is limited to make an affirmative remark on solutions having a scanty literature available. There has been a debate regarding the ideal time effect of each chelating agent. Even with the vast amount of research on the topic, there is no clear defined irrigation protocol. There have been disagreements regarding the ideal chelator and the application time. The amount of time these solutions stay in contact with the canal walls has reportedly varied from 1 to 15 minutes. [23, 24] In the present study, we have used a 5-minute exposure time for irrigation on the dentin surface for each chelating solution. [25, 26]

Other than SEM, the smear layer can also be scored by using digital image analysis. It can overcome the potential of evaluator bias, requires less time, and other parameters of interest like density and average diameter of dentinal tubules can be measured (27), but SEM was opted in this study because it is a commonly available tool for evaluating the smear layer. The samples used in this study are single-rooted anterior teeth with relatively straight canal. Thus, our results may be limited to only such clinical cases. Further studies using digital image analysis can be conducted to evaluate whether a similar

effect of citric acid and deferoxamine can be obtained in multirouted teeth with curved canals

CONCLUSION

Within the limitations of the study it can concluded that Citric acid can be used as an alternative irrigant to 17% EDTA as it dissolves organic and inorganic tissues and has less adverse effects.

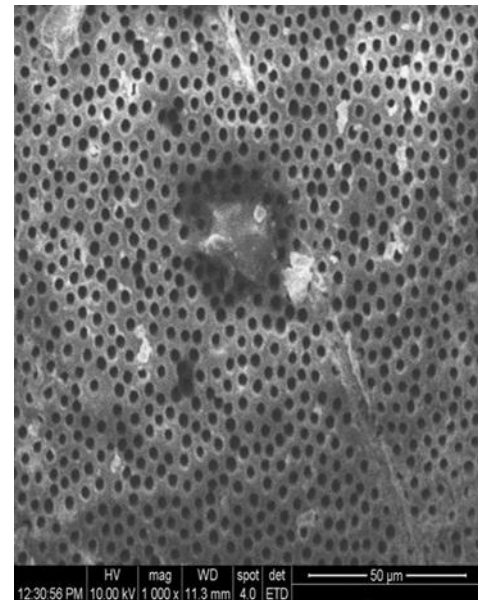


Fig.1: Smear layer removal by EDTA

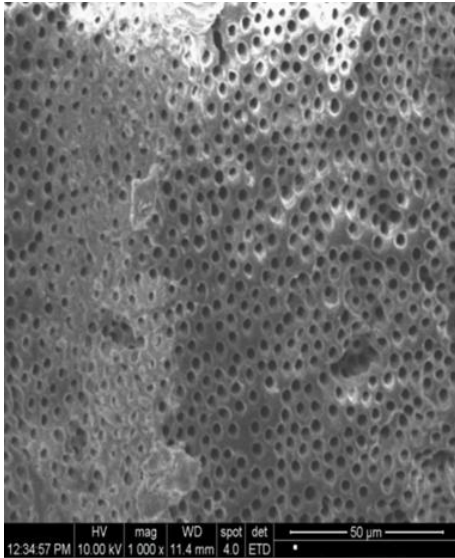


Fig. 2: Smear layer removal by citric acid

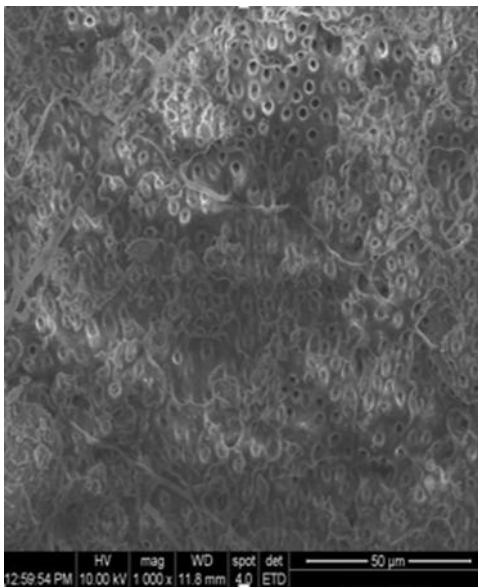


Fig.3: smear layer removal by Deferoxamine

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