



A Comparative Study on Dentinal Tubule Penetration of Endodontic Irrigants: Confocal Laser Microscopy Insights

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/jammr/2024/v36i115633>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/126065>

Original Research Article

Received: 28/08/2024

Accepted: 31/10/2024

Published: 04/11/2024

ABSTRACT

Aim: This *In vitro* study is aimed to analyse and compare the dentinal penetration depth of three endodontic irrigants using Confocal laser microscope.

Study Design: It is a descriptive study.

Methodology: 17%EDTA, 17%REDTA and 17% Citric Acid at acidic pH (pH5.5) and distilled water as a control was taken as final root canal irrigants. 60 human permanent single rooted teeth were taken from a pool of extracted teeth, decoronated and mechanically shaped using waveone gold file

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system and apex was prepared by WaveOne Gold Large (size 0.45, taper 0.07) file and 5.25% sodium hypochlorite solution. The final rinsing was done with rhodamine B dye conjugated EDTA, REDTA, Citric acid and distilled water. After this, teeth were embedded in epoxy resin and sectioning was done at coronal, middle and apical third level using microtome and thin sections of approx.200µm thickness were prepared. Sections were examined under confocal laser microscope and statistical analysis was done using ANOVA and Post-Hoc Tests.

Results: Study revealed that the citric acid is the best endodontic irrigant having maximum dentinal penetration depth at all three levels.

Keywords: EDTA; REDTA; citric acid; dentinal penetration depth; pH; confocal laser microscope.

1. INTRODUCTION

“The prognosis of a root canal treatment depends on many factors and one of the important factors is an endodontic irrigant chosen. An ideal solution is a non-viscous, non-irritating, germicidal solution with detergent qualities and abilities to dissolve necrotic tissue” [1]. “The antibacterial effect, achieved by endodontic treatment, is more likely affected by degree of the penetration of irrigants to scavenge bacteria residing deeply inside infected dentinal tubules than by instrumentation of the root canal system because the shaping protocol revealed deficient debridement and area untouched by both manual K-files and rotary or reciprocating instruments” [2]. “The calcium present in hydroxyapatite crystals is one of the main inorganic elements of dentin. Any change in the calcium ratio can significantly alter the original proportion of organic and inorganic components, which can alter dentin permeability, micro-hardness, and solubility” [3]. “EDTA has self-limiting action, forms a stable with calcium and dissolve dentin. Citric acid is used in concentrations ranging from 1- 40% in endodontic practice to remove smear layer after root canal preparation. 10% citric acid have been proven to be more effective in removing smear layer and dentine dissolution when compared with EDTA and also has antimicrobial effects” [4-6]. In order to increase the cleaning and bactericidal potential of the solution, detergent can be added to EDTA. Another advantage of this addition is reducing the surface tension of the irrigant, facilitating the wetting of the entire root canal wall and thereby increasing the ability of the chelators to penetrate the root canal dentin [7-9]. Kennedy et al. [10] reported that once the smear layer was removed by using REDTA (EDTA 17.00 g, cetyl trimethylammonium bromide 0.84 g, 5N sodium hydroxide solution 9.25 mL, and distilled water 100.00 mL), dentinal openings could be created more easily in younger teeth than in physiologically older teeth,

especially in more sclerotic middle and apical thirds. A literature review has revealed that different mixtures, concentrations, pH, working time and methods have been used to evaluate the irrigating solutions [11]. Thus considering these factors, this study will be conducted to evaluate in vitro the penetration depth into dentinal tubules of 17%EDTA, 17%REDTA, 17% Citric Acid and distilled water (control) at acidic pH.

2. MATERIALS AND METHODOLOGY

This in vitro study is done by using final irrigants namely 17% EDTA (Waldent Alchem), lab formulated 17% citric acid (Rankem), lab formulate 17% REDTA (EDTA 17.00 g, cetyl trimethylammonium bromide 0.84 g ((Loba chemicals), 5N sodium hydroxide solution 9.25 mL (Rankem), and distilled water 100.00 mL) and distilled water and pH 5.5 was adjusted on pH meter by addition of Hydrochloric acid on digital pH meter (decibel) at department of Pharmacology, World College of Medical Sciences & Research and Hospital (WCMSRH), Jhajjar (Haryana).

60 human permanent teeth with single, straight, oval-shape root canal with fully formed apices were chosen from a pool of extracted teeth and were stored in 5% sodium hypochlorite solution (Septodont) for 2 hrs for soft tissue dissolution and then the teeth were stored in .9% saline solution (Hi Line-NS) till their use. Then the crowns were removed by diamond disc. Then the adequacy of the working length (WL) and the presence of an open apical foramen were confirmed by inserting a size 10 K-file (Mani). A mechanical glide path was established using

WaveOne Gold Glider reciprocating single files (Densply). Subsequently, each root canal was shaped using WaveOne Gold Medium (size 0.35, taper 0.06) followed by WaveOne Gold Large (size 0.45, taper 0.07) files. The instruments

were employed with a slow in-and-out pecking motion using an Eighteenth endomotor. The WL was reassessed with a size 10 K-File. "Following each instrument change, each canal was irrigated with 1 mL of 5.25% NaOCl (Safe Endo) using a syringe equipped with a 30-gauge side-vented needle. The needle was positioned at least 2 mm away from the working length (WL). A total of 10 mL of 5.25% NaOCl was used for each specimen. Following irrigation, the outer surface of the teeth was dried using paper towels. Then, a thin layer of dual composite was applied to cover the apical third of the teeth. After that the samples were randomly segregated into 4 distinct experimental groups. After that, the samples were finally rinsed with 5ml of each 17% EDTA, 17% REDTA, 17% citric acid and distilled water using 30-gauge side vent needle. Each solution was labelled with .1%wt rhodamine B dye and was left for 3minutes and then the canals were dried with Wave One Gold paper points of large size. After final irrigation, in laboratory (Lab Crystals, Aliganj, Lucknow, Uttar Pradesh), the teeth were embedded in methyl-methacrylate resin. After hardening the resin, 200µm transverse sections of teeth were cut at coronal, middle and apical third by microtome and slides were prepared. After fixing the slides, the slides were analysed under Confocal Laser Microscope Leica (TCS SP8C) at 10X using FIJI software and then data was analysed in the

Microsoft Excel 2007 and using the SPSS statistical software 23.0 Version (Department of Cell Biology, IIT Sonipat (a branch of Central Research Facility IIT Delhi), Haryana)" [2].

3. RESULTS

The mean dentinal penetration depth (in µm) was shown maximum by citric acid group and least was shown by distilled group (Table 1). The maximum penetration depth at coronal, middle and apical was also topped by citric acid group and at bottom was distilled water group. Confocal images of various groups at various levels are shown in Table 2.

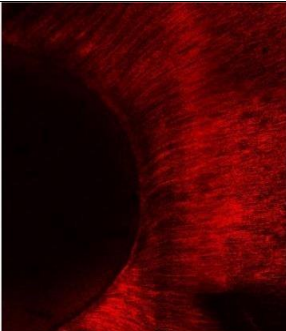
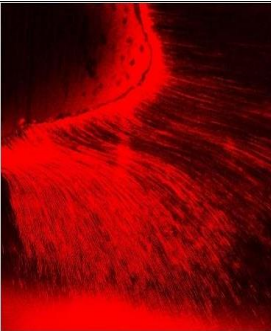
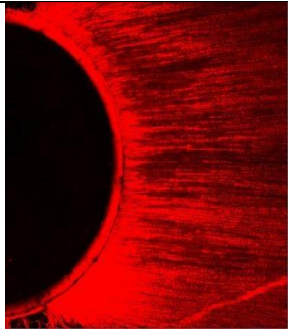
3.1 Statistical Analysis

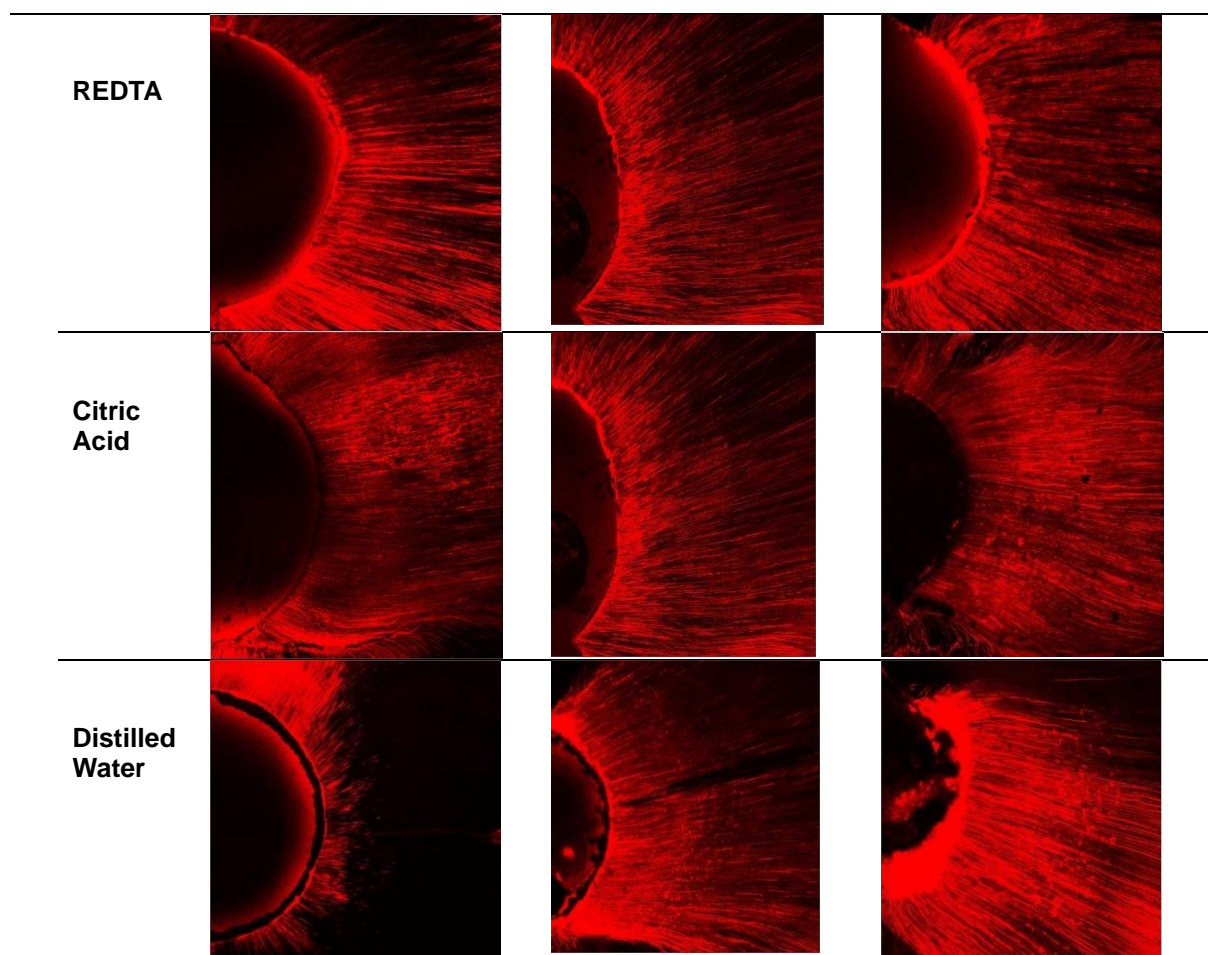
The data was entered in the Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. The descriptive statistics included mean, standard deviation frequency and percentage. The level of the significance for the present study was fixed at 5%. The intergroup comparison was done using the One Way ANOVA test followed by post Hoc Analysis depending upon the normality of the data. The Shapiro–Wilk test was used to investigate the distribution of the data and Levene’s test to explore the homogeneity of the variables.

Table 1. Comparison of mean (in µm) among four groups

| | Apical | Middle | Coronal |
|-----------------------|---------------|---------------|----------------|
| Group Distilled Water | 200.01±40.684 | 325.02±52.371 | 534.50±54.527 |
| Group REDTA | 293.31±43.018 | 544.78±56.139 | 824.90±54.605 |
| Group EDTA | 366.71±44.978 | 785.33±56.413 | 1216.40±51.247 |
| Group Citric Acid | 593.31±41.018 | 976.96±58.384 | 1648.00±60.593 |

Table 2. Confocal laser microscopic images of various groups at various level

| Group Name | Coronal Section | Middle Section | Apical Section |
|------------|---|--|---|
| EDTA |  |  |  |



4. DISCUSSION

In the present in vitro study, the penetration depth of four irrigating solutions at acidic pH (EDTA, REDTA, Citric Acid and distilled water as control) into dentine tubules is evaluated. The three irrigants used in the study except distilled water are chelating agents. This study indicates higher penetration depths into dentinal tubules in the coronal and middle thirds of the teeth from all the groups and a lower penetration depth is in the apical third of all the root canals studied, confirming findings of previous studies [12].

The present findings might be attributed to the influence of the root canal system's anatomical structure on the depth to which irrigants can penetrate into dentinal tubules. Indeed, dentinal tubules in the apical third of the root canals are less permeable than those in the coronal and middle thirds due to tubular sclerosis, the smaller diameter, and the reduced number [13]. The diameter of dentine tubules decreases from 1.2 μm at the pulp-dentine junction to 0.4 μm at cemento-dentinal junction [14]. The number of

tubules per square millimetre is also greater near to pulp (58000 mm^{-2}) than further away from the pulp (10000 mm^{-2}) [15]. As the tubules density reduces towards the apex, so does the dentine permeability [16]. Moreover, root dentine is not mineralised uniformly. Apical dentine is more sclerosed and mineralised [17]. Dentine permeability is directly related to area of tubule lumina and indirectly to wall thickness of the root canal [18]. After BMP, wall thickness is reduced and surface area of the root canal is increased but the smear layer acts as a diffusion barrier, reducing dentine permeability by 25-49% [19-22].

Luciano Giardino [23-31], Generali, L. [32] and Peters OA [33] reported that lower surface tension enables the irrigant to penetrate into dentinal tubules, microscopic irregularities, and accessory canals. Taşman et al. [34] reported that on observing the average surface tension values of the test solutions, it was noted that Ringer's solution, saline solution, and distilled water—which are known to lack any chemical effect—had the highest values, questioning once again the efficacy of these solutions in

endodontic therapy. On the other hand, the relatively lower surface tension values of NaOCl and EDTA may contribute to the high success rates achieved with the combined use of these irrigants as reported in previous reports. Abbaszadegan et al. [35] and La Rosa GRM*, Scolaro C, Leanza G et al. [36] also supported the high surface tension of distilled water, limiting the penetration depth into dentinal tubules. Dushan B. Naumovich [37] showed the highest surface tension of distilled water amongst 22 endodontic irrigants used in the experiment and supported March, T. [38], who stated that Along with osmotic activity and diffusibility (free diffusion), surface tension is considered one of the most important factors that determine the penetrability and spreading of drug. These above statements support the least penetration of distilled water into dentinal tubules.

In this study, 2nd last dentinal penetration depth was obtained from REDTA group. REDTA is a chelating agent, formulated by addition of 0.84g Cetyl-trimethylammonium bromide (Cetrimide) to 17%EDTA in aqueous medium and buffered by NaOH at pH 8 and then made acidic (pH 5.5) by addition of HCl. Cetrimide {cetyltriethyl ammonium bromide (CTAB)} is both a disinfecting agent and a cationic surfactant, reduces the surface tension of the irrigant [26,39-40], improves antibacterial effectiveness [40-42], facilitates penetration of the irrigant to the dentin surface [43], and increases the wettability of the dentin surface [26]. In addition, cetrimide may also alter the structure of hydroxyapatite nano rods in a concentration dependent manner. It also elongates the hydroxyapatite nano rods, thereby the length diameter ratio of these nano rods decreases when the content of CTAB increases potentially altering the physical properties of dentin [44]. In 1991, Sterrett JD, Delaney B, Rizkalla A, Hawkins CH stated that the cationic surfactants are potent antimicrobial agents that have also been shown to act on biofilm components, but they have no decalcifying effects on root canal dentin [44]. Then again in 2011 by Tianyuan M, Zhigou X, Libing L. [45] reported that this low penetration might be due to the demineralizing action of chelating agents results in increased exposure of organic content. This organic matrix of dentine acts as a limiting factor for further dissolution of inorganic content, thus reducing the decalcifying action of chelating solutions over time. Again in 2014, same findings were supported by Poggio C, Dagna A, Colombo M, Scribante A, Chiesa M. by stating that the presence of cetrimide in the

irrigating solutions does not improve the extraction of Ca²⁺ from root dentine and it could be considered useful to complete antibacterial activity of irrigating solutions [46]. La Rosa GRM*, Scolaro C, Leanza G et al. [36] reported that there were no significant changes reported in surface tension and wetting ability regarding EDTA group and EDTA PLUS group (EDTA containing surface active compounds). As we consider that low surface tension results in high dentinal penetration, so from above statement it can be derived that EDTA plus might be resulted in low penetration depth into dentinal tubules as we analysed in our study. Many previous studies also reported the similar findings [26,47]. Giardino L, Ambu E, Becce C, Rimondini L, Morra M [23] had stated that the actors affecting the depth of penetration of root canal irrigants could be surface tension, viscosity and molecular size, so another reason of low dentinal penetration depth might be increased viscosity due to addition of surfactants in to EDTA.

The chelation effect typically involves the formation of multiple coordinate bonds between a multidentate ligand (electron pair donor) and a metal ion (electron pair acceptor). EDTA, a hexaprotic weak acid (H₆Y²⁺) featuring four carboxylic acids and two ammonium groups, was initially suggested by Nygaard-Ostby for use in root canal preparation through chelation. When mixed with water, EDTA exhibits an acidic pH but cannot be utilized directly for irrigation due to its poor solubility. Moreover, the mechanism of EDTA's action involves both protonation and chelation, which vary depending on the pH level., [48,10,49]. At a low pH, the protonation of EDTA reduces the number of available electron pairs, which in turn slows down the dissociation of hydroxyapatite (HAp) and the process of demineralization. Conversely, at high or neutral pH levels, EDTA's ability to bind calcium ions promotes the dissociation of HAp, making it more available for chelation. To improve EDTA's water solubility while reducing the protonation effect, its pH must be adjusted to at least 7. EDTA chelates calcium ions, forming soluble calcium chelates. When disodium EDTA is added to the system, it removes calcium ions from the solution by forming these soluble chelates [50] Ballal NV [51], Torabinejad M et al. [52] and Mancini M [53] revealed EDTA to be less successful in the apical third compared with the coronal and middle thirds for the removal of SL. It has been shown that the dentinal tubules progressively sclerose in the apical third [13,54]. Thus, the activity of EDTA may not be as effective in the

apical third [53]. Calve, Medina, and Shnche [29] has reported the almost similar findings. Cury JA⁵⁵ reported that the efficiency of EDTA solutions can be achieved between a pH of 5 to 6. However, EDTA preparations usually have an average pH of 7.3, possibly exerting a greater solubility effect on hydroxyapatite. At high pH values, the excess number of hydroxyl groups will slow down the dissociation of hydroxyapatite, thus limiting the number of available Ca²⁺. At a low or neutral pH, the binding of Ca²⁺ will tend to increase the dissociation of hydroxyapatite and its availability for chelation [56,57]. Yilmaz et al. [26] showed that plain EDTA solution may only be suitable for wetting of dentin at a pH of 5.5 in 37°C. It might be suggested that using EDTA solution during root canal treatment might result in better surface tension level because the body temperature is around 37°C. "Furthermore, the ineffectiveness of NaOCl and Ethylenediaminetetraacetic acid (EDTA) in dissolving smear layer in the apical third of root canals can attribute to their high dynamic viscosity [41]. Moreover, Because the wettability of pure EDTA itself is poor, some reports have suggested that a surfactant should be added to pure EDTA solution" [58-65]. Further, Ravnik C [66] and Cury JA [55] stated that as pH decreases, the solutions might not be so penetrating.

"An alternative to EDTA, which is less widely recognized but still possesses strong chelating properties, is citric acid" [25,67-68]. "Its ability to bind to metal ions makes it valuable in processes where the removal or sequestration of metals is necessary. Therefore, EDTA" [48] and citric acid [69] have been recommended as adjuvants in root canal therapy. The 17% citric acid is used in this study because in addition to its biocompatibility, it has the ability to eliminate inorganic smear layer tissue. This is consistent with Schafer's [70] assertion that 1–40% citric acid can be used in root canal treatment because it is effective in removing smear layers, in dissolving the dentin powder and in demineralizing the intertubular dentin to open the dentinal tubules. Additionally, this is in accordance with research by Malheiros [71] showing that 17% EDTA has higher cytotoxicity than 10%, 15%, and 25% citric acid, so 10–25% citric acid had better biocompatibility than 17% EDTA. Sousa S.M., S. T [3] has conducted a study to biochemically compare the decalcifying effects of 1% EDTA (pH 7.4), 1% EGTA (pH 7.4), 1% CDTA (pH 7.4), 1% citric acid solutions (pH 1.0 and 7.4) and saline solution (control) on root

dentin and reported that 1% citric acid at pH 1.0 was the best solution to remove calcium from the root dentin. Taking the inspiration from their study, this study is also aimed to compare the dentinal penetration depth making the similar concentrations and pH of the root canal irrigants. This study reveals that the maximum penetration depth into dentinal tubules is shown by 17% citric acid at pH 5.5. long-standing studies reported its best action at lower pH [71-72]. Both studies have demonstrated that the pH level of a citric acid solution is a more crucial factor for demineralization than its concentration. Sterrett et al. [44] suggested that "this phenomenon might result from a balance between the decreasing pH and the increasing viscosity of the solution as the constituent concentration rises. At high concentrations, citrate occupies a significant portion of the solvent, greatly reducing the amount of solvent available for Ca²⁺ diffusion". Previous literature has been suggested that 10% citric acid has been as a good alternative to EDTA for better elimination of the smear layer from the root canal walls [67,65,28]. The reason may be its acidic pH, which increases the elimination of calcium [46].

5. CONCLUSION

This is the first study aimed to compare and analyse dentinal penetration depth using the 17% citric acid, 17% EDTA and 17% REDTA as an endodontic irrigants at pH 5.5 and distilled water as a control. In spite of limitations of this study, this research reports the 17% citric acid as a better root canal irrigant than EDTA, REDTA and distilled water and it also shows that the addition of a surfactant to EDTA has little effect on dentinal penetration.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as ChatGPT (Free version, GPT 4o mini, downloaded from Google Chrome) was used to just frame the title only. No other addition was done using AI technology.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

FUNDING

This research is self-financed by the main author.

DATA AVAILABILITY STATEMENT

The data is available with the main author on request.

ACKNOWLEDGEMENTS

I would like to thank Department of Pharmacology, World College of Medical Sciences & Research and Hospital (WCMSRH), Jhajjar (Haryana) for irrigants formulation, Lab Crystals, Aliganj, Lucknow (Uttar Pradesh) for teeth sectioning and slide preparation & fixation and Department of Cell Biology, IIT (a branch of Central Research Facility IIT Delhi) Sonipat (Haryana) for slide analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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