

Effects of EDTA, fumaric acid, and the chitosan solutions prepared with distilled water and acetic acid on dentin microhardness and tubular penetration: An in vitro study

Esma Sariçam^{1,A–D,F}, Hanife Altınışık^{2,A–C,E,F}, Neslihan Bulak^{3,B,C,F}, Berna Güven^{4,B,C,E,F}, Sabiha Zeynep Yurdağül^{5,B,F}, Gülin Amasya^{6,B,F}

¹ Department of Endodontics, Faculty of Dentistry, Ankara Yıldırım Beyazıt University, Turkey

² Department of Restorative Dentistry, Faculty of Dentistry, Gazi University, Ankara, Turkey

³ Private practice, Ankara, Turkey

⁴ Department of Pharmacology, Faculty of Pharmacy, Ankara University, Turkey

⁵ Fatma Kemal Timuçin Oral and Dental Hospital, Adana, Turkey

⁶ Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2025;62(4):731–737

Address for correspondence

Esma Sariçam

E-mail: dt.esmasaricam@gmail.com

Funding sources

None declared

Conflict of interest

None declared

Acknowledgements

None declared

Received on April 11, 2023

Reviewed on May 18, 2023

Accepted on June 6, 2023

Published online on March 28, 2025

Cite as

Sariçam E, Altınışık H, Bulak N, Güven B, Yurdağül SZ, Amasya G. Effects of EDTA, fumaric acid, and the chitosan solutions prepared with distilled water and acetic acid on dentin microhardness and tubular penetration: An in vitro study. *Dent Med Probl.* 2025;62(4):731–737. doi:10.17219/dmp/167467

DOI

10.17219/dmp/167467

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the

Creative Commons Attribution 3.0 Unported License (CC BY 3.0)

(<https://creativecommons.org/licenses/by/3.0/>).

Abstract

Background. Chitosan prepared with acetic acid is commonly used as an endodontic irrigant. However, the chitosan solution prepared with distilled water has not been evaluated for endodontic usage.

Objectives. The present study aimed to compare the effects of ethylenediaminetetraacetic acid (EDTA), fumaric acid, and the chitosan solutions prepared with distilled water (C-DW) and acetic acid (C-AA) on dentin microhardness and dentinal tubule penetration.

Material and methods. Eighty maxillary central incisors were endodontically instrumented and randomly divided into 2 main groups ($n = 40$) for the evaluation of dentin microhardness and tubular penetration, with 4 subgroups in each main group ($n = 10$) according to the final irrigation solutions used (EDTA, fumaric acid, C-DW, and C-AA). The C-AA solution was prepared by diluting medium-molecular-weight chitosan in acetic acid. The C-DW solution was prepared with distilled water and chitosan ammonium salts, which were synthesized using trichloroacetic acid and low-molecular-weight chitosan. After irrigation, the roots were sectioned horizontally 2 mm (the apical third) and 5 mm (the middle third) from the apex. The microhardness measurements were taken at depths of 500 μm and 1,000 μm from the canal lumen. The sections were examined for tubular penetration using confocal laser scanning microscopy. The data was analyzed using the analysis of variance (ANOVA), with a significance level set at $p < 0.05$.

Results. The microhardness values were statistically similar at either depth for each third ($p > 0.05$), except for the 1,000- μm depth in the apical third, where the use of the C-AA solution resulted in lower microhardness as compared to fumaric acid ($p < 0.05$). No significant differences were observed in tubular penetration with regard to each third ($p > 0.05$).

Conclusions. All solutions showed a similar penetration ability in each third. At the 1,000- μm depth in the apical third, the fumaric acid solution provided a higher microhardness value than the C-AA solution.

Keywords: chitosan, chelating agents, confocal laser scanning microscopy, fumaric acid

Highlights

- All the tested irrigation solutions (EDTA, fumaric acid, chitosan prepared with distilled water (C-DW), and chitosan prepared with acetic acid (C-AA)) demonstrated comparable ability to penetrate dentinal tubules in both the apical and middle thirds of the root canals.
- While the microhardness values were similar across most conditions, fumaric acid showed superior preservation of dentin microhardness at the 1,000- μ m depth in the apical third as compared to C-AA.
- C-DW and C-AA performed similarly in terms of tubular penetration and microhardness, indicating that C-DW is a viable alternative to C-AA for clinical use in root canal treatment.

Introduction

The success of endodontic treatment depends on the effective chemo-mechanical preparation and three-dimensional (3D) filling of the root canal system.¹ Chemo-mechanical preparation aims to shape and clean the root canals. Mechanical preparation is insufficient to reach root canal irregularities and dentinal tubules, and it cannot eliminate microorganisms from those untouched surfaces.² Mechanical preparation promotes the formation of the smear layer, which consists of organic and inorganic tissue remnants, microorganisms, and their by-products.³ The smear layer negatively affects the penetration of irrigation solutions and the adhesion of the filling material.⁴ The irrigation solutions used for chemical preparation mainly aim to reach and eliminate microorganisms in the uninstrumented areas, and dissolve the components of the smear layer.⁵ The efficiency of irrigants is affected by their ability to penetrate into dentinal tubules, the irrigation method and root canal structures.⁶

Irrigation solutions may act similarly on the smear layer and root canal dentin.⁷ Irrigants can cause changes in the chemical and mechanical properties of dentin by affecting its mineral content.⁸ The determination of microhardness shows mineral changes in root canal dentin.⁹ A decreased microhardness value may indicate the dissolution or deterioration of dentin composition,¹⁰ which adversely affects the adhesion of the sealer.¹¹

Several irrigation solutions are preferred for smear layer removal, e.g., ethylenediaminetetraacetic acid (EDTA), maleic acid, QMix®, MTAD, fumaric acid, and chitosan.^{7,12–14} EDTA is the most widely used irrigation solution for this purpose. Irrigation with a 0.7% concentration of fumaric acid has been considered efficient in smear layer removal.¹³ Fumaric acid is a trans isomer of maleic acid and is produced in a citric acid cycle.¹⁵ Its esters have been successfully used for the treatment of psoriasis and multiple sclerosis.¹⁶

Recently, 0.2% chitosan has been applied in irrigation procedures.¹⁷ Chitosan is a natural polysaccharide obtained from crab and shrimp shells.¹⁸ Its use in the biomedical and dentistry fields is highly popular due to its good biocompatibility, biodegradability, antimicrobial properties, and chelating activity toward metal ions.¹⁹

The 0.2% chitosan solution prepared using medium-molecular-weight chitosan and acetic acid (C-AA) has been found effective in removing the smear layer from root canal walls.²⁰ However, chitosan dissolves in acidic conditions and has poor water solubility, which limits its further industrial applications.²¹ To overcome this drawback, chitosan derivatives have been synthesized from halogens and low-molecular-weight chitosan to provide good water solubility. These chitosan ammonium salts are considered to have better antifungal and antibacterial properties than chitosan itself.^{21,22} The chitosan solution with distilled water (C-DW) is prepared by dissolving chitosan ammonium salts in distilled water.

Previously, EDTA and 0.2% chitosan solutions were compared with regard to dentin microhardness changes and the ability of the irrigant to penetrate into dentinal tubules.⁴ A literature review showed that fumaric acid and the C-DW solution had not been evaluated in terms of their effects on dentin microhardness and tubular penetration ability. Thus, the present study aimed to compare the effects of different final irrigation solutions, including EDTA, fumaric acid, and the C-DW and C-AA solutions, on dentinal tubule penetration capacity and dentin microhardness changes. The null hypothesis stated that no differences would be observed in the effects of various irrigants on dentinal tubule penetration and dentin microhardness.

Material and methods

This study was approved by the ethics committee of Ankara Yıldırım Beyazıt University, Turkey (research code: 2020-149; decision date and No. of approval: 09.07.2020/39). Eighty extracted human maxillary central incisor teeth with full apex formation were selected. The teeth were visually and radiographically examined to have one single root and root canal, and to have no caries, resorptions or calcifications. The teeth were decoronated at the cemento-enamel junction (CEJ) under water coolant to obtain a 17-mm root length. The working length was determined as 16 mm. The root canals were prepared using the ProTaper Next X4 instruments (Dentsply Maillefer, Ballaigues, Switzerland) with an endodontic

electronic torque-controlled motor. The root canals were irrigated with 2 mL of 2.5% NaOCl irrigation solution at each instrument change. The roots were equally divided into 2 main groups to evaluate the effects of the tested irrigants on dentin microhardness and dentinal tubule penetration. The tested irrigants were as follows: EDTA; fumaric acid; C-DW; and C-AA.

Preparation of irrigation solutions

The EDTA solution: 17% EDTA solution was used.

The fumaric acid solution: The solution was prepared at a 0.7% concentration by mixing fumaric acid with distilled water.

The C-DW solution: Chitosan ammonium salts with halogens were prepared according to the method of a previous study,²² using trichloroacetic acid and low-molecular-weight chitosan. The solution was prepared by dissolving the chitosan-trichloroacetate compound in distilled water at a concentration of 5 mg/mL.

The C-AA solution: Chitosan with a deacetylation degree of 70–85% (Sigma-Aldrich, St. Louis, USA) was dissolved in 1% acetic acid solution to obtain 0.2% chitosan solution.⁷ The acetic acid solution was added to the weighed amount of chitosan and the mixture was stirred overnight with the help of a magnetic stirrer. Then, the solution was filtrated through Whatman 42 filter paper to remove the undissolved material.

Evaluation of dentin microhardness

The 40 roots were randomly divided into 4 subgroups ($n = 10$) for microhardness evaluation: EDTA; fumaric acid; C-AA; and C-DW. The roots were irrigated using 5 mL of the solution for 1 min. Afterward, irrigation with 5 mL of distilled water was performed to prevent the residual action of the solutions on dentin. The roots were embedded into acrylic resin blocks. The roots were sectioned vertically along the long axis under distilled water coolant, using a low-speed saw (Micracut 151; Metkon Instruments Inc., Bursa, Turkey), and 2.0-millimeter-thick slices were obtained at 2 mm (the apical third) and 5 mm (the middle third) from the root apex.

A Vickers microhardness testing machine (HNV-700; Shimadzu Corporation, Tokyo, Japan) was used at a load of 100 g for 10 s. Three indentations were made at 500 μm and 1,000 μm from the canal lumen, with 100 μm between the indentations, under a stereomicroscope integrated into the microhardness testing machine at $\times 40$ magnification. The arithmetic mean of the Vickers microhardness values was calculated for each distance.

Evaluation of dentinal tubule penetration

All final irrigants were mixed with 0.1% fluorescent rhodamine B isothiocyanate (Merck, Darmstadt, Germany)

to visualize dentinal tubules under confocal laser scanning microscopy. Root surfaces were sealed with nail polish. The apical foramina of the roots were covered with wax. The group was randomly divided into 4 subgroups ($n = 10$) according to the final irrigation solution: EDTA; fumaric acid; C-AA; and C-DW. All groups were irrigated with 5 mL of the solution for 1 min. The roots were embedded in acrylic resin and sectioned vertically along the long axis under distilled water coolant, using a low-speed saw (Micracut 151). At 2 mm (the apical third) and 5 mm (the middle third) from the root apex, 1.0-millimeter-thick slices were obtained. The coronal sides of the slices were polished with silicon carbide abrasive paper. The samples were fixed on glass coverslips, with the apical sides of the slices contacting the coverslips.

The specimens were scanned and photographed using a confocal laser scanning microscope (Zeiss LSM 510; Carl Zeiss, Jena, Germany) under the Ar/HeNe laser excitation, with a wavelength of 543 nm (Fig. 1). The images were evaluated using the ImageJ software, v. 1.48 (<https://imagej.net/ij>; National Institutes of Health (NIH), Bethesda, USA), to analyze the irrigant penetration areas. In each image, the outline of the penetration area and the circumference of the root canal were marked. The percentage of irrigant penetration was calculated by dividing the outlined area by the root canal area.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). The normal distribution of the data in each group was evaluated using the Shapiro–Wilk test. The data showing a normal distribution was evaluated using the one-way analysis of variance (ANOVA), post-hoc Tukey's test and t test. The significance level was set at $p < 0.05$.

Results

Evaluation of dentin microhardness

The results regarding Vickers microhardness are shown in Table 1. No significant differences were found between the tested irrigants in the 500- μm and 1,000- μm depth microhardness values for the middle third ($p > 0.05$), and in the 500- μm depth microhardness values for the apical third ($p > 0.05$). C-AA provided a statistically significantly lower microhardness value than fumaric acid at the 1,000- μm depth for the apical third ($p < 0.05$). Regardless of the irrigant used, the irrigation procedure resulted in a statistically significantly lower microhardness values in the middle third than in the apical third at either depth ($p < 0.05$) (Table 2).

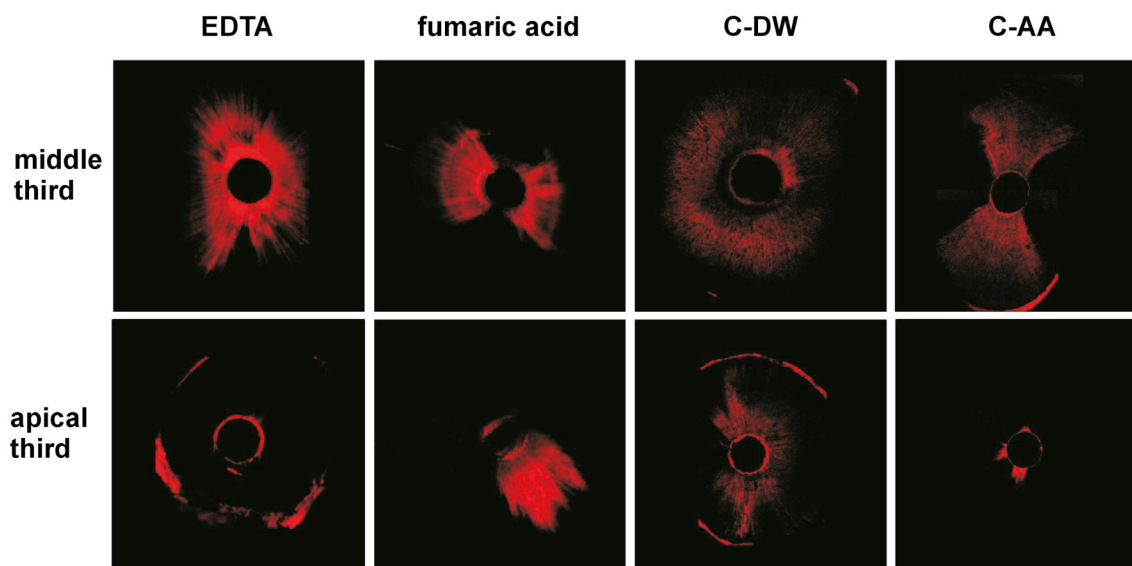


Fig. 1. Representative confocal laser scanning microscopic images for each irrigation solution at the middle and apical thirds

EDTA – ethylenediaminetetraacetic acid; C-DW – chitosan prepared with distilled water; C-AA – chitosan prepared with acetic acid.

Table 1. Comparison of the irrigants with regard to the Vickers microhardness values obtained at depths of 500 μ m and 1,000 μ m from the canal lumen for the apical and middle thirds of the root canals

Irrigant	Apical third						Middle third					
	500 μ m			1,000 μ m			500 μ m			1,000 μ m		
	Vickers microhardness [HV]	F	p-value	Vickers microhardness [HV]	F	p-value	Vickers microhardness [HV]	F	p-value	Vickers microhardness [HV]	F	p-value
EDTA (n = 10)	76.99 $\pm$ 9.38			83.33 $\pm$ 6.42 ^{ab}			75.56 $\pm$ 7.62			82.22 $\pm$ 3.75		
Fumaric acid (n = 10)	76.57 $\pm$ 5.12	1.601	0.206	86.01 $\pm$ 3.66 ^a	5.405	0.004*	75.47 $\pm$ 4.61	1.436	0.248	83.98 $\pm$ 4.52	1.620	0.202
C-DW (n = 10)	76.04 $\pm$ 4.85			82.08 $\pm$ 3.78 ^{ab}			77.10 $\pm$ 6.25			81.65 $\pm$ 3.34		
C-AA (n = 10)	71.71 $\pm$ 3.43			78.27 $\pm$ 2.81 ^b			70.87 $\pm$ 9.14			78.87 $\pm$ 8.08		

Data presented as mean $\pm$ standard deviation ($M \pm SD$).

* statistically significant (ANOVA and post hoc Tukey's test). Different superscript letters indicate significant differences within each column.

Table 2. Comparison of the apical and middle thirds with regard to the dentin microhardness values and the irrigant penetration ratios, regardless of the type of irrigant

Part of the root canal	500 μ (n = 40)			1,000 μ (n = 40)			Penetration ratio (n = 40)	t	p-value
	Vickers microhardness [HV]	t	p-value	Vickers microhardness [HV]	t	p-value			
Apical third	78.52 $\pm$ 6.94	2.382	0.020*	82.42 $\pm$ 5.07	0.054	0.003*	7.94 $\pm$ 4.97	2.690	0.009*
Middle third	74.75 $\pm$ 7.22			78.49 $\pm$ 6.38			11.61 $\pm$ 7.03		

Data presented as $M \pm SD$.

* statistically significant (t test).

Evaluation of dentinal tubule penetration

No significant differences were observed between the tested irrigants in terms of dentinal tubule penetration in the apical and middle thirds ($p > 0.05$) (Table 3).

The penetration ratio obtained in the middle third was statistically higher than that in the apical third, regardless of the irrigant used ($p < 0.05$) (Table 2).

Table 3. Comparison of the irrigants with regard to the percentage of dentinal tubule penetration

Irrigant	Apical third			Middle third		
	dentinal tubule penetration [%]	F	p-value	dentinal tubule penetration [%]	F	p-value
EDTA (n = 10)	7.625 ±5.864	0.629	0.601	11.497 ±5.556	2.435	0.081
Fumaric acid (n = 10)	6.687 ±3.083			8.321 ±4.890		
C-DW (n = 10)	9.699 ±5.491			10.477 ±6.351		
C-AA (n = 10)	7.764 ±5.251			16.128 ±9.107		

Data presented as $M \pm SD$ (ANOVA).

Discussion

An irrigation solution should have the abilities of smear layer removal and dentinal tubule penetration without promoting a decrease in dentin microhardness. In this study, the effects of the EDTA, fumaric acid, C-AA, and C-DW solutions on dentin microhardness and dentinal tubule penetration were evaluated. The effects of the solutions in terms of dentinal tubule penetration were similar in the apical and middle thirds of the root canals. Dentin microhardness values for the solutions were similar at the 500- μm and 1,000- μm depths in the middle third, and at the 500- μm depth in the apical third. However, in the apical third, C-AA showed a lower microhardness value than fumaric acid at the 1,000- μm depth. Thus, the null hypothesis was partially accepted.

The dentin microhardness measurement is a non-destructive method that indirectly proves mineral loss or gain in dental hard tissues.⁹ A positive correlation has been proven between dentin mineralization and microhardness.²³ The Vickers indenter method has been found to be more sensitive for evaluating deep dentin microhardness than the Knoop method.²⁴ In this study, Vickers indentation was preferred.

Chitosan irrigation solutions prepared with different concentrations were previously evaluated. Chitosan solutions diluted with acetic acid at 0.2% and 0.5% concentrations were similar in terms of the Ca/P ratio changes and smear layer removal in root canal dentin.²⁵ Chitosan solutions at 0.37% and 0.2% concentrations were similar with regard to removing the smear layer, but 0.37% chitosan caused a much greater erosive effect.²⁶ Therefore, in this study, the 0.2% concentration was preferred for the preparation of the C-AA solution. A 0.7% concentration of fumaric acid was used, as this concentration represents the maximum dissolution rate of fumaric acid at room temperature.¹³

The chelating feature of EDTA reduces dentin microhardness. EDTA causes demineralization by binding the Ca ions of dentin, thus softening the intraradicular dentin.²⁷ The effect of the 0.2% chitosan solution prepared

with medium-molecular-weight chitosan and acetic acid on dentin microhardness was found to be similar to that of 17% EDTA solution,^{7,28} which is in accordance with the results of this study. Supporting these results, the C-AA solution prepared with acetic acid and the C-DW solution prepared with distilled water similarly affected dentin microhardness in each third and at either depth in this study. The chelating mechanism of chitosan is not yet clear, but the chelating process is considered to occur between the amino group of chitosan and metallic ions.⁷ Using acetic acid or distilled water for a chitosan solution similarly affected dentin microhardness. Further studies should be conducted to evaluate the effect of C-AA and C-DW on the physical and chemical properties of dentin.

Regardless of the irrigant used, higher microhardness values were found in the apical third at either depth. The intraradicular dentin in the apical third was more sclerosed and the concentration of non-collagenous proteins decreased. Thus, the level of demineralization in the apical third area of the root canal also decreased.²⁹ In the apical third at the 1,000- μm depth, fumaric acid showed the highest microhardness value, although the difference was statistically significant only in comparison with C-AA. Fumaric acid has also been found to be more effective in smear layer removal than EDTA due to 2 carboxylic groups opposite each other in its molecular form, which provides a greater binding ability to Ca ions.¹³ In this study, fumaric acid also showed a similar penetration ability to that of other irrigation solutions.

Scanning electron microscopy (SEM) is a widely used method to evaluate dentinal tubule penetration. However, this technique has some drawbacks, such as the requirement for the vacuum and gold sputtering procedures, promoting artifacts, a limited number of analyzed surfaces, and the subjective examination of images.³⁰ Confocal laser scanning microscopy does not require any specimen preparation and provides fewer artifacts.³¹ In the present study, confocal laser scanning microscopy was used for the evaluation of irrigant penetration. A fluorescent dye, rhodamine B, in a low concentration (0.1%) was used to detect the irrigant. The sections were obtained 2 mm and 5 mm from the apical foramen, as observed in previous studies.^{32–34}

Regardless of the irrigant used, tubular penetration was lower in the apical third, as dentinal tubules are more sclerosed and fewer there than in the middle third.³⁵

Better penetration of the irrigant can be achieved by better smear layer removal. The similar penetration ability of the solutions shows that they have a similar smear layer removal ability. The dentinal tubule penetration of irrigation solutions not only improves the flow of the sealer, but also provides better antimicrobial activity. The dentin wettability depends on the viscosity and surface tension of the irrigant, which both affect the tubular penetration of the irrigants.³⁶ Similar mean values of tubular penetration were observed for all irrigation solutions, regardless of the root canal third assessed. The tested solutions could also have similar viscosity. Further studies comparing the viscosity patterns of these solutions would be beneficial. In the apical third, C-DW showed a better penetration ability than C-AA. C-DW was prepared with low-molecular-weight chitosan. The lower weight may provide a deeper diffusion ability, thus enabling the irrigant to penetrate deeper into the narrow tubules involved in the apical third.

The 0.2% chitosan solution prepared with medium-molecular-weight chitosan and acetic acid was evaluated for the smear layer removal effect. A chitosan solution prepared with low-molecular-weight chitosan and distilled water has not been evaluated as an irrigation solution in the literature. The C-DW solution manifested similar penetration ability, as well as the microhardness values at the 500- μ m and 1,000- μ m depths, as the C-AA solution in each third. However, the lowest microhardness values at the 1,000- μ m depth were observed for the C-AA solution. Therefore, the C-DW solution can be considered an alternative to the C-AA solution.

Conclusions

All the tested irrigation solutions showed a similar tubular penetration ability in the apical and middle thirds. The microhardness values were also similar for the 500- μ m depth for each third. At the 1,000- μ m depth in the apical third, the fumaric acid solution provided an increased microhardness value as compared to the C-AA solution.

Ethics approval and consent to participate

The study was approved by the ethics committee of Ankara Yıldırım Beyazıt University, Turkey (research code: 2020-149; decision date and No. of approval: 09.07.2020/39).

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Esma Sariçam  <https://orcid.org/0000-0001-7701-4214>
 Hanife Altınışık  <https://orcid.org/0000-0001-7430-4750>
 Neslihan Bulak  <https://orcid.org/0000-0002-2621-4395>
 Berna Güven  <https://orcid.org/0000-0001-9181-3741>
 Sabiha Zeynep Yurdağül  <https://orcid.org/0000-0001-9444-7281>
 Gülin Amasya  <https://orcid.org/0000-0002-0491-450X>

References

- Caron G, Nham K, Bronnec F, Machtou P. Effectiveness of different final irrigant activation protocols on smear layer removal in curved canals. *J Endod.* 2010;36(8):1361–1366. doi:10.1016/j.joen.2010.03.037
- Ricucci D, Siqueira JF Jr. Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. *J Endod.* 2010;36(1):1–15. doi:10.1016/j.joen.2009.09.038
- Violich DR, Chandler NP. The smear layer in endodontics – a review. *Int Endod J.* 2010;43(1):2–15. doi:10.1111/j.1365-2591.2009.01627.x
- Silva Antunes PV, Souza Flamini LE, Mazzi Chaves JF, Silva RG, da Cruz Filho AM. Comparative effects of final canal irrigation with chitosan and EDTA. *J Appl Oral Sci.* 2020;28:e20190005. doi:10.1590/1678-7757-2019-0005
- Prado M, Gusman H, Gomes BP, Simão RA. Scanning electron microscopic investigation of the effectiveness of phosphoric acid in smear layer removal when compared with EDTA and citric acid. *J Endod.* 2011;37(2):255–258. doi:10.1016/j.joen.2010.11.011
- van der Sluis LW, Gambarini G, Wu MK, Wesselink PR. The influence of volume, type of irrigant and flushing method on removing artificially placed dentine debris from the apical root canal during passive ultrasonic irrigation. *Int Endod J.* 2006;39(6):472–476. doi:10.1111/j.1365-2591.2006.01108.x
- Saha SG, Sharma V, Bharadwaj A, et al. Effectiveness of various endodontic irrigants on the micro-hardness of the root canal dentin: An in vitro study. *J Clin Diagn Res.* 2017;11(4):ZC01–ZC04. doi:10.7860/JCDR/2017/24018.9472
- Panighi M, G'Sell C. Effect of the tooth microstructure on the shear bond strength of a dental composite. *J Biomed Mater Res.* 1993;27(8):975–981. doi:10.1002/jbm.b.820270802
- Arends J, ten Bosch JJ. Demineralization and remineralization evaluation techniques. *J Dent Res.* 1992;71 Spec No:924–928. doi:10.1177/002203459207100527
- Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. *J Dent.* 1999;27(1):43–46. doi:10.1016/s0300-5712(98)00018-9
- Perdigão J, Eiriksson S, Rosa BT, Lopes M, Gomes G. Effect of calcium removal on dentin bond strengths. *Quintessence Int.* 2001;32(2):142–146. PMID:12066675.
- Nogo-Živanović D, Kanjevac T, Bjelović L, Ristić V, Tanasković I. The effect of final irrigation with MTAD, QMix, and EDTA on smear layer removal and mineral content of root canal dentin. *Microsc Res Tech.* 2019;82(6):923–930. doi:10.1002/jemt.23239
- Jaiswal S, Patil V, Satish Kumar KS, Ratnakar P, Rairam S, Tripathi S. Comparative analysis of smear layer removal by conventional endodontic irrigants with a newly experimented irrigant-fumaric acid: A scanning electron microscopic study. *J Conserv Dent.* 2018;21(4):419–423. doi:10.4103/JCD.JCD_290_16
- Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: A scanning electron microscopic study. *J Endod.* 2009;35(11):1573–1576. doi:10.1016/j.joen.2009.07.021

15. Yazdi MR, Mrowietz U. Fumaric acid esters. *Clin Dermatol*. 2008;26(5):522–526. doi:10.1016/j.clindermatol.2008.07.001
16. Gkalpakiotis S, Arenberger P, Gkalpakioti P, Meluzinova E, Chandran D, Arenbergerova M. Management of psoriasis vulgaris and multiple sclerosis with fumaric acid. *J Am Acad Dermatol*. 2014;70(3):e60–e61. doi:10.1016/j.jaad.2013.10.031
17. Zhou H, Li Q, Wei L, Huang S, Zhao S. A comparative scanning electron microscopy evaluation of smear layer removal with chitosan and MTAD. *Niger J Clin Pract*. 2018;21(1):76–80. doi:10.4103/1119-3077.224798
18. Akncbay H, Senel S, Ay ZY. Application of chitosan gel in the treatment of chronic periodontitis. *J Biomed Mater Res B Appl Biomater*. 2007;80(2):290–296. doi:10.1002/jbm.b.30596
19. Aguilar A, Zein N, Harmouch E, et al. Application of chitosan in bone and dental engineering. *Molecules*. 2019;24(16):3009. doi:10.3390/molecules24163009
20. Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. *Int Endod J*. 2013;46(4):332–338. doi:10.1111/j.1365-2591.2012.02119.x
21. Guo Z, Xing R, Liu S, et al. Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydr Res*. 2007;342(10):1329–1332. doi:10.1016/j.carres.2007.04.006
22. Tan W, Li Q, Dong F, Wei L, Guo Z. Synthesis, characterization, and antifungal property of chitosan ammonium salts with halogens. *Int J Biol Macromol*. 2016;92:293–298. doi:10.1016/j.ijbiomac.2016.07.023
23. Panighi M, G'Sell C. Influence of calcium concentration on the dentin wettability by an adhesive. *J Biomed Mater Res*. 1992;26(8):1081–1089. doi:10.1002/jbm.820260809
24. Fuentes V, Toledano M, Osorio R, Carvalho RM. Microhardness of superficial and deep sound human dentin. *J Biomed Mater Res A*. 2003;66(4):850–853. doi:10.1002/jbm.a.10064
25. Mathew SP, Pai VS, Usha G, Nadig RR. Comparative evaluation of smear layer removal by chitosan and ethylenediaminetetraacetic acid when used as irrigant and its effect on root dentine: An in vitro atomic force microscopic and energy-dispersive X-ray analysis. *J Conserv Dent*. 2017;20(4):245–250. doi:10.4103/JCD.JCD_269_16
26. Silva PV, Costa Guedes DF, Pécora JD, da Cruz-Filho AM. Time-dependent effects of chitosan on dentin structures. *Braz Dent J*. 2012;23(4):357–361. doi:10.1590/s0103-64402012000400008
27. Akcay I, Sen BH. The effect of surfactant addition to EDTA on microhardness of root dentin. *J Endod*. 2012;38(5):704–707. doi:10.1016/j.joen.2012.02.004
28. Pimenta JA, Zapparolli D, Pécora JD, Cruz-Filho AM. Chitosan: Effect of a new chelating agent on the microhardness of root dentin. *Braz Dent J*. 2012;23(3):212–217. doi:10.1590/s0103-64402012000300005
29. Kawasaki K, Ruben J, Stokroos I, Takagi O, Arends J. The remineralization of EDTA-treated human dentine. *Caries Res*. 1999;33(4):275–280. doi:10.1159/000016529
30. Machado R, da Fonseca Roberti Garcia L, da Silva Neto UX, da Cruz Filho AdM, Silva RG, Vansan LP. Evaluation of 17% EDTA and 10% citric acid in smear layer removal and tubular dentin sealer penetration. *Microsc Res Tech*. 2018;81(3):275–282. doi:10.1002/jemt.22976
31. Kok D, Húngaro Duarte MA, Da Rosa RA, Wagner MH, Pereira JR, Reis Só MV. Evaluation of epoxy resin sealer after three root canal filling techniques by confocal laser scanning microscopy. *Microsc Res Tech*. 2012;75(9):1277–1280. doi:10.1002/jemt.22061
32. Akcay M, Arslan H, Mese M, Durmus N, Capar ID. Effect of photon-initiated photoacoustic streaming, passive ultrasonic, and sonic irrigation techniques on dentinal tubule penetration of irrigation solution: A confocal microscopic study. *Clin Oral Investig*. 2017;21(7):2205–2212. doi:10.1007/s00784-016-2013-y
33. Generali L, Cavani F, Serena V, Pettenati C, Righi E, Bertoldi C. Effect of different irrigation systems on sealer penetration into dentinal tubules. *J Endod*. 2017;43(4):652–656. doi:10.1016/j.joen.2016.12.004
34. Küçük M, Kermeoğlu F. Efficacy of different irrigation methods on dentinal tubule penetration of chlorhexidine, QMix and Irritrol: A confocal laser scanning microscopy study. *Aust Endod J*. 2019;45(2):202–208. doi:10.1111/aej.12309
35. Paqué F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. *Int Endod J*. 2006;39(1):18–25. doi:10.1111/j.1365-2591.2005.01042.x
36. Bukiet F, Soler T, Guivarch M, et al. Factors affecting the viscosity of sodium hypochlorite and their effect on irrigant flow. *Int Endod J*. 2013;46(10):954–961. doi:10.1111/iej.12086