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Effect of Maleic Acid, Ethylendiaminetetraacetic Acid, MTAD on Smear Layer Removal and Dentin Microhardness

Natasha Gupta
Department of Conservative Dentistry, Jamia Millia Islamia, New Delhi, India, natasshagupta@gmail.com

Neelam Singh
Department of Conservative Dentistry, Jamia Millia Islamia, New Delhi, India

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ORIGINAL ARTICLE

Effect of Maleic Acid, Ethylendiaminetetraacetic Acid, MTAD on Smear Layer Removal and Dentin Microhardness

Natasha Gupta, Neelam Singh

Department of Conservative Dentistry, Jamia Millia Islamia, New Delhi, India
Correspondence e-mail to: natasshagupta@gmail.com

ABSTRACT

Objective: In this study, we aimed to compare efficacy of various irrigating solutions for smear layer removal and dentin microhardness. **Methods:** Based on the four final irrigants used plus saline control, 50 single-rooted teeth were divided into five groups. Using a step back technique with K files, chemomechanical preparation was performed. Canals were apically enlarged up to ISO size 40 and stepped back up to ISO size 60. During preparation, irrigation was performed with 2.5% NaOCl solution and the roots were sectioned into two halves. In the coronal, middle, and apical thirds, the smear layer was evaluated by scanning electron microscopy in one half, whereas the dentin microhardness was evaluated in the other half. **Results:** For all irrigants in the coronal and middle third regions, the efficacy of smear layer removal was comparable. Doxycycline, citric acid, Tween 80 (MTAD) and 10% maleic acid were the most effective for the apical third region, followed by 7% maleic acid and 17% ethylenediaminetetraacetic acid (EDTA). Dentin microhardness was most affected by MTAD and 10% maleic acid, followed by 17% EDTA and 7% maleic acid. **Conclusion:** For removal of smear layer and the least effect on dentin microhardness, 7% maleic acid was effective.

Key words: dentin microhardness; ethylenediaminetetraacetic acid; maleic acid. MTAD; smear layer removal

Natasha Gupta, Neelam Singh. Effect of maleic acid, ethylendiaminetetraacetic acid, MTAD on smear layer removal and dentin microhardness. J Dent Indones. 2018;25(2): 91-8.

INTRODUCTION

Endodontic therapy's success depends on a thorough biomechanical preparation, which can help achieve a 3D obturation.[1] This therapy's final outcome is successfully providing a tight hermetic apical and coronal seal. When thorough cleaning and shaping are performed by instrumentation and irrigation, 3D obturation is possible. Instrumentation tends to leave an amorphous, granular layer that covers the root dentin, which contains both organic and inorganic material, and has been described as the smear layer by McComb and Smith.[2] This layer's formation cannot be avoided whose presence tends to block dentinal tubules.[3] If this layer is not removed, it eventually is degraded by proteolytic enzymes released by bacteria and lead to an incomplete seal and microleakage.[3] Thus, this layer's removal improves the adaptation of obturating materials to canal walls.[4] To flush the debris and remove the smear layer, various irrigating solutions and techniques, such as distilled water, saline, hydrogen peroxide, sodium hypochlorite (NaOCl), ultrasonics, and electrochemically activated water, have been used during and after instrumentation.[5] For removing the smear layer, chelating agents are considered to be the most superior as they form calcium chelate solutions with calcium ions of dentin, thereby making dentin more friable and easier to instrument.[1,2] Note that the smear layer has very small particles with a large surface–mass ratio, making them soluble in acids.[6] Currently, ethylenediaminetetraacetic acid (EDTA) is the most commonly used agent.[7] The combination of 17% EDTA and 5.25% NaOCl effectively removes the smear layer for the coronal and middle one-third regions but not for the apical one-third region.[7]

Dentin microhardness is sensitive towards composition and surface changes in tooth structure and the multiple chemical irrigants that are used tend to decrease microhardness. These changes may have a profound effect on the tooth's strength.[5]

In this study, we evaluated and compared 17% EDTA, MTAD (a mixture of doxycycline, citric acid, and a detergent [Tween 80]), and 7% and 10% maleic acid for smear layer removal of the coronal, middle, and apical third of root canals as well as the effect of each irrigant on dentin microhardness.

METHODS

Fifty periodontally involved human maxillary central incisors that had single roots, which were devoid of caries, cracks, endodontic treatment, or restorations, were selected with the approval of the Institutional Ethical Committee of the Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India. Using a gauze piece and a fine brush, the soft tissue covering the root surface was removed. For a maximum of 2 months, the teeth were stored in 0.2% sodium azide at 4°C. Later, to obtain standardized root lengths of 15 mm, the teeth were decoronated at the cemento-enamel junction using a diamond disc under a water coolant. The working length was measured with a size 10 K file (Dentsply Maillefer, Baillagues, Switzerland). Using a step back technique with the K files, biomechanical preparation was performed. The canals were apically enlarged up to ISO size 40, and step back was completed up to ISO size 60. During canal preparation, irrigation was performed with 2.5% NaOCl solution (Prime Dental, Thane, India). Based on the final irrigant used, the teeth were then randomly divided into five groups (n = 10). Five milliliters of each irrigant was used and left in the canal for 2 min. The test solutions that were used were 17% EDTA (Smear Clear, Sybron Endo), 7% and 10% maleic acid (Merck), MTAD (Biopure, Dentsply), and saline control. The irrigation method was standardized using 28-gauge side-vented needles. The needle penetration's depth was 14 mm. The experimental groups were group 1, 17% EDTA; group 2, 7% maleic acid; group 3, 10% maleic acid; group 4, MTAD; and group 5, saline. Maleic acid was prepared as an irrigant at the Department of Pharmacology, Jamia Millia Islamia.

For each group, teeth were longitudinally sectioned by making grooves on the labial and palatal sides without cutting through canals. Then, the teeth were split apart with a sharp chisel. The tooth's one half was used to measure dentin microhardness, whereas the other half was subjected to scanning electron microscopy (SEM) examination for analyzing the smear layer at the apical, middle, and coronal one-third.

The other half of sectioned root was subjected to Scanning electron microscope for evaluation of smear layer.

SEM Analysis

The samples were dehydrated and washed in a series of 50%, 70%, 90%, and 95% ethanol mixtures and twice in absolute ethanol for 30 min. The samples were loaded on brass stubs with carbon tapes, and then they were sputter-coated with gold after placing them on a copper grid for 3 to 5 min. They were then viewed under a scanning electron microscope (JSM-6390A, Analytical SEM, Jeol, Tokyo, Japan), which was operated in secondary electron mode at 1000× and 3000× magnification at an accelerating voltage of 25 kV.

Table 1. Criteria of the appearance of smear layer

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

The scoring of Smear layer was done according to the According to guidelines suggested by Torabinejad in 2003, the smear layer was scored for which each specimen was precalibrated by two observers. An interrater agreement was measured between two observers by having both observers evaluate half of images for each of the two separate sessions.

The smear layer' presence on the root canal's surface or in dentinal tubules at the coronal, middle, and apical portion of each canal was coded based on the following the criteria in Table 1.

Microhardness of Root Canal Dentin

Sectioned roots were horizontally mounted on autopolymerizing acrylic resin, forming a block while the dentin surface was exposed. Microhardness was measured by subjecting them to Vickers microhardness testing under 100× magnification using a 200-g load for 25 s dwell time. The root sections were then divided into three equal regions, coronal, middle, and apical and three readings were taken for each region.

RESULTS

The data are presented as mean and standard deviation of scores obtained for smear layer removal by observers 1 and 2 for all the three regions of the root. The mean values that are presented are inversely proportional to efficacy of irrigants for smear layer removal (Tables 2 and 3). Using a Kruskal–Wallis one-way analysis of variance (ANOVA), statistical comparison was performed with $p < 0.05$ considered to indicate statistical significance. A post-hoc Tukey HSD test was performed to compare all the scores from both observers for the different study groups (Table 4).

In the coronal and middle one-third regions, smear layer removal was comparable for all groups, with no significant differences existing between the groups. For both observers, there were statistically significant differences between scores for apical one-third regions: group 4 (10% maleic acid) = group 3 (MTAD) > group 2 (7% maleic acid) > group 1 (17% EDTA) > group 5 (saline) (Figures. 1–5).

Table 2. Scanning electron micrograph scores: observer 1. Apical one-third

17% EDTA group 1	Maleic acid 7% group 2	Maleic acid 10% group 3	MTAD group 4	Saline group 5
2	2	2	1	3
3	3	2	2	3
1	2	2	1	3
2	3	2	1	3
2	2	2	1	3
3	2	2	2	3
3	3	1	1	3
2	2	1	1	3
2	2	2	1	3
2	2	1	2	3

EDTA, ethylenediaminetetraacetic acid; MTAD, mixture of doxycycline, citric acid, and a detergent (Tween 80).

Table 3. Scanning electron micrograph scores: observer 2. Apical one-third

17% EDTA group 1	Maleic acid 7% group 2	Maleic acid 10% group 3	MTAD group 4	Saline group 5
3	3	2	3	3
3	2	2	2	3
2	2	1	1	3
2	3	2	1	3
2	1	1	3	3
3	2	2	2	3
3	3	1	1	3
2	2	2	2	3
3	2	2	1	3
2	2	2	3	3

EDTA, ethylenediaminetetraacetic acid; MTAD, mixture of doxycycline, citric acid, and a detergent (Tween 80)

Table 4. Multiple comparisons of the apical one-third observation

(I) Irrigants	(J) Irrigants	Observer 1			Observer 2		
		Mean difference	SE	p	Mean difference	SE	p
Saline	17% EDTA	0.700*	0.206	0.016	0.800*	0.255	0.032
	Maleic acid 10%	1.300*	0.206	0.000	1.300*	0.255	0.000
	MTAD	1.700*	0.206	0.000	1.500*	0.255	0.001
	Maleic acid 7%	0.800*	0.206	0.004	0.500*	0.255	0.379
17% EDTA	Saline	–.700*	0.206	0.016	–.800*	0.255	0.032
	Maleic acid 10%	1 .000*	0.206	0.056	0.500	0.255	0.379
	MTAD	0.000*	0.206	0.000	0.700	0.255	0.847
	Maleic acid 7%	0.100	0.206	0.997	–.300	0.255	0.847
Maleic acid 10%	Saline	–1.300*	0.206	0.000	–1.300*	0.255	0.000
	17% EDTA	–.500	0.206	0.167	–.800*	0.255	0.032
	MTAD	0.400	0.206	0.391	–.200	0.255	0.969
	Maleic acid 7%	–.500	0.206	0.167	–.800*	0.255	0.032
MTAD	Saline	–1.700*	0.206	0.000	–1.100*	0.255	0.001
	17% EDTA	–.900	0.206	0.001	0.600	0.255	0.193
	Maleic acid 10%	–.400	0.206	0.391	0.200	0.255	0.969
	Maleic acid 7%	–.900	0.206	0.001	–.600	0.255	0.193

EDTA, ethylenediaminetetraacetic acid; MTAD, mixture of doxycycline, citric acid, and a detergent (Tween 80).
Dependent variable, Tukey HSD

Table 5. Apical one-third: intraclass correlation coefficient

Measure	Intraclass[a] correlation	95% CI		*F* test with true value 0			
		Lower bound	Upper bound	Value	Df1	Df2	*p*
Single measures	.630[b]	.450	.762	4.412	59.0	59	.000
Average measures	.773[c]	.621	.865	4.412	59.0	59	.000

Two-way mixed effects model in which people-related effects are random and measure-related effects are fixed.
[a]Type C intraclass correlation coefficients using a consistency definition; the between-measures variance is excluded from the denominator variance.
[b]The estimator is the same, whether the interaction effect is present or not.
[c]This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Table 6. The results of the microhardness test

Group	*n*	Total (3 indentations)	Mean	SD
Group 1: 17% EDTA	10	43.6460	1.63658	.51753
Group 2: 7% maleic acid	10	46.3970	1.65389	.52301
Group 3: 10% maleic acid	10	46.6270	2.19302	.69350
Group 4: MTAD	10	40.6260	1.5832	.49974
Group 5: Saline	10	62.5610	1.56263	.49415

EDTA, ethylenediaminetetraacetic acid; MTAD, mixture of doxycycline, citric acid, and a detergent (Tween 80).

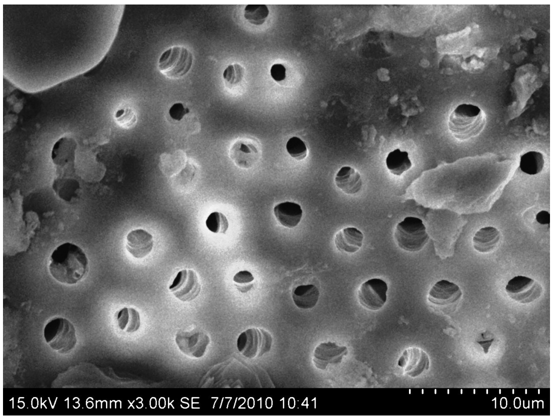

Figure 1. Scanning electron micrograph of the apical one-third region of the group 1 tooth.

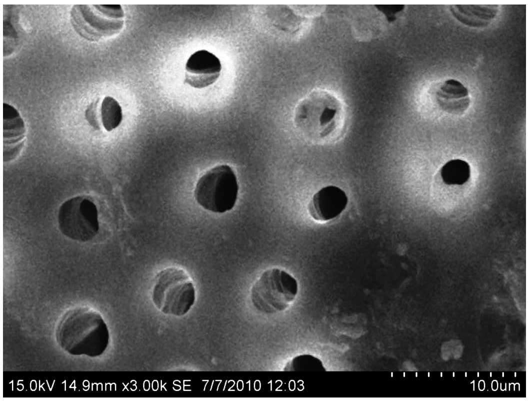

Figure 2. Scanning electron micrograph of the apical one-third region of the group 2 tooth.

To assess the reliability of observers, an interclass correlation coefficient test was performed. Interclass correlation values of >0.6 were considered to indicate reliability. Because the data were quantitative, Kappa correlation was not used. Moreover, since the interclass correlation coefficient value was >0.6 for all three regions, the scores for both observers were considered to be reliable (Table 5).

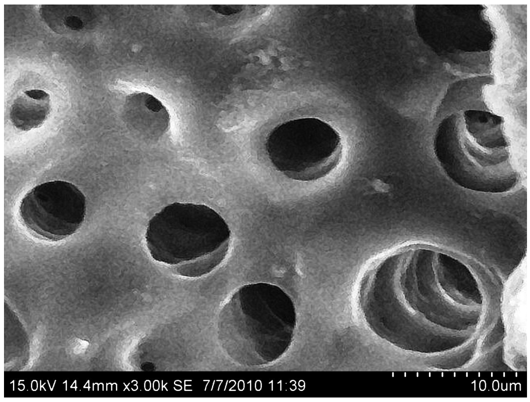

Figure 3. Scanning electron micrograph of the apical one-third region of the group 3 tooth.

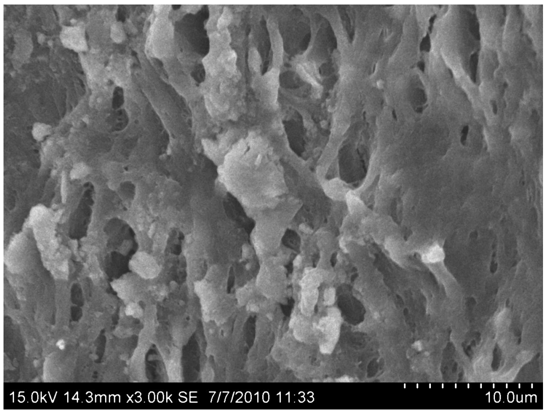

Figure 4. Scanning electron micrograph of the apical one-third region of the group 4 tooth.

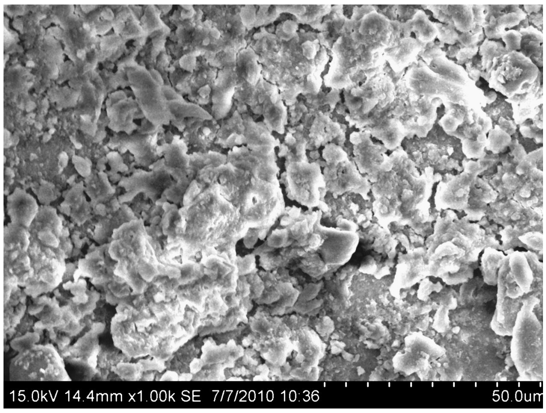

Figure 5. Scanning electron micrograph of apical the one-third region of the group 5 tooth.

Analysis of Dentin Microhardness

For each sample, the results of the dentin microhardness test are presented as a mean of three values (Table 6). The results showed group 3 (MTAD) < group 4 (10% maleic acid) < group 1 (17% EDTA) = group 2 (7% maleic acid).

DISCUSSION

For the development of periapical and pulpal lesions, microbes play a crucial role;[8] therefore, it is extremely important to completely eradicate them. A combination of instrumentation and irrigation is very important for success in endodontic therapy.[8] The irrigant's effectiveness in removal of smear layer from the root canal depends on the following factors: the irrigant's aggressiveness and the manner in which it is delivered,[10] chemical nature and quantity of solution, contact time, penetration depth of the irrigating needle, type and gauge of the needle, surface tension of the irrigating solution, and the solution's age.[5]

For this study, the teeth were cleaned and stored in 0.2% sodium azide for a month. This helped maintain sterile conditions prior to the experimental procedures as mentioned in previous studies too.[13] The step back technique was utilized because dentin, which is composed of hydroxyapatite, tends to smear when abraded with a hard instrument. This leads to formation of sludge material, which is known as the smear layer.[3] The layer's thickness was determined by the type and sharpness of cutting instruments along with dentin's water content. An increase in centrifugal forces resulted in the movement and proximity of instruments to the dentinal wall. This resulted in a thicker and more resistant smear layer; thus, the the smear layer's volume produced by rotary instrumentation was greater than that produced by hand instrumentation.[1]

To allow adequate cleaning and penetration of irrigating solution, the apical portion of each canal was prepared up to size 30 file.[14] This is in accordance with other studies in which larger apical preparations produced a greater reduction in the remaining bacteria and dentin debris.[15] The delivery method for the irrigant was standardized using a side-vented needle provided with MTAD such that the irrigant was not extruded beyond the apical foramen. To ensure that the irrigant reached the apex, the needle was inserted to a premeasured depth of 14 mm.[16] The effectiveness of various irrigating solutions in removing the smear layer was evaluated by SEM because SEM is found to be a more reliable tool for evaluating smear layers and examining morphological details of the prepared root canals' surface.[17] For unbiased results, two observers scored the SEM images.

Among the irrigants that were used in the coronal and middle third regions, there were no significant differences in the smear layer's removal, whereas a smear layer's presence was observed in the few remaining groups at the apical end. This could be attributed to the morphological variations of the root canal system, particularly at the apical third, which has numerous ramifications, accessory canals, lateral canals, and apical deltas that may prevent complete debridement of the apical third. Other influencing factors include sclerotic[16] dentin and reductions in the number and diameter of dentinal tubules from the coronal to the apical third.[18] Furthermore, the tubule's orifices are enlarged because of dissolution of the peritubular dentin, which has a lower collagen content that increases the speed of dissolution for acids.[19]

In this study, for the final rinse, 2.5% NaOCl was followed by 17% EDTA; however, the choice of final irrigant is debatable. Certain studies favor NaOCl as the final irrigant,[11] while other studies recommend a chelator followed by chlorhexidine as the final irrigant.[5] When NaOCl is used for the final rinse after EDTA, EDTA can chemically interact with NaOCl and reduce the amount of freely available chlorine. This potentially inhibits the antibacterial activity and tissue-dissolving potential of NaOCl.[20] This is particularly true with resin sealers or resin-based obturation because NaOCl inhibits the resin's polymerization.[21] Moreover, it significantly reduces the hardness, flexural strength, and modulus of elasticity of dentin.[22]

Similar to the results obtained by Ciucchi et al., 17% EDTA was not very effective for removing the smear layer in the apical third region in our study.[37] The decrease in EDTA's efficacy in the apical region could be because of a decrease in the noncollagenous organic matrix's content because the EDTA solution reduces both the mineral and noncollagenous protein components of dentin.[6] In previous studies, 17% EDTA was found to be effective when in contact with the root canal for 1 to 5 min;[23] however, in our study, 2 minutes' contact yielded satisfactory results. Furthermore, the efficacy of 17% EDTA decreases with time,[6] which may be because all available ions get bound and an equilibrium is established; thus, there is no further dissolution.[6] While EDTA removes inorganic components of the smear layer, leaving behind fibrous components,[24] the organic matrix of the dentin accumulates on canal walls, thereby preventing further dissolution.[24] Such a demineralization procedure will continue until all the available chelators form complexes composed of calcium.[6] This self-limiting action may be because of pH changes during dentin's demineralization.

BioPure MTAD, which contains a tetracycline isomer 3%, doxycycline 150 ml/5 ml, 4.25% citric acid, a detergent, and Tween 80, has been introduced in endodontics.[28] It is an acidic solution with a pH of 2.15, which makes it capable of removing inorganic substances.[29] The present study's results showed that MTAD was the most effective irrigant for removing smear layers from the three regions of the root canal, confirming the results previously obtained.[17,20] In these studies, it was claimed that MTAD was capable of disinfecting dentin, removing the smear layer, opening dentinal tubules, and allowing antibacterial agents to penetrate the complete root canal system.[17] The demineralization potential could be attributed to tetracycline's presence, which is bacteriostatic in nature, along with the unique property of having a low pH ; therefore, it acts as a chemical chelator similar to citric acid.[17]

Our study demonstrated that MTAD was more effective at the apical third region compared to 17% EDTA. The reason could be a more aggressive nature compared with 17% EDTA for demineralizing the intact intraradicular dentin and exposing the collagen matrix, which is 1.5 to 2 times thicker.[30] Moreover, after irrigation with MTAD, a 10-µm-thick zone of demineralized dentin is created on the dentin surface.[30] This zone may be formed because of the slow degradation of the collagen matrix, which occurs via the release and activation of endogenous matrix metalloproteins (MMPs) from partially demineralized dentin.[30] In this manner, MTAD provides a sustained MMP-inhibitory function. Furthermore, by chelating zinc and calcium EDTA also inhibits MMP activity; however, it does not exhibit a sustained inhibition toward MMPs released subsequently from underlying mineralized dentin,[30] which makes it less efficient. Tween 80's presence, which reduces surface tension of irrigants, may also increase the irrigant's efficacy at the apical end. Furthermore, reducing the irrigant's surface tension improves its dentin-wetting ability and enhances flow into narrow canals, thereby providing an intimate contact between irrigants and dentinal walls of root canals.[31]

In this study, two concentrations of maleic acid, 7% and 10%, were used. At the apical third region, the efficacy of 10% maleic acid was similar to that of MTAD compared with other irrigants. The capability of maleic acid to remove the smear layer and demineralize intertubular dentin was because of its pH of 1.05.[13] Our observations demonstrated that reduction of microhardness by 10% maleic acid was similar to that demonstrated in a study by Prabhu et al.[32]

Moreover, our results demonstrated that 7% maleic acid was more effective compared to 17% EDTA for removing smear layers from the apical third, a result similar to that obtained by Ballal et al.[33] This may be because the surface tension of 17% EDTA (0.078 N/m) is greater than that of 7% maleic acid (0.063 N/m); moreover, maleic acid, is more acidic and thus has a greater demineralizing effect.[33]

By valuating microhardness, you can obtain indirect evidence of mineral loss or gain for dental hard tissue.[33] In this study, we determined microhardness by a Vickers microhardness tester. In previous studies, for evaluating surface changes of dental hard tissue treated with chemical irrigants, the suitability and practicality of this tester has been compared with the Knoop hardness tester.[33] The dentin hardness characteristically increases from the root canal lumen toward the cementodentinal junction, whereas the values in the apical one-third are lower than those in the middle and cervical sections of the root.[6] Because dentin's microhardness may vary considerably within the same tooth[34] and may decrease as the indentations that are tested are closer to the pulp,[34] the hardness was measured at the 0.5-mm level from root canal spaces for standardization.

In this study, three values were considered for standardization and the mean for each sample was calculated. The decrease in microhardness as we reach close to the pulp could be explained by increase in the number of widely opening dentinal tubules that are free of peritubular dentin near the pulp, which offers little resistance to the testing indenter.[35] The unaffected root dentin's hardness is between 40 and 75 kg mm^{-2}; moreover, chelators can change the root dentin hardness by 20 VHN (Vickers hardness).[6] To simulate clinical conditions, the samples were not immersed in the irrigants; however, the effect of each test solution when used as an irrigant was evaluated.

We identified a decrease in the dentin's microhardness when 17% EDTA was used for 2 min. This result may be because of the exposure of radicular dentin to EDTA for >1 min, which may cause erosion of both peritubular and intertubular dentin.[19] Furthermore, it was confirmed that EDTA decalcified dentin to a depth of 20–40 µm in 5 min,[5] which may be a contributing factor to remnants of smear layers and reduction in the microhardness that was observed.

We identified a decrease in dentin microhardness by irrigation with MTAD, a result similar to that obtained by Saghiri et al. This could be because of the 3% doxycycline hyclate component of MTAD, which acts as a calcium chelator and causes demineralization. However, there was no significant difference between the microhardness of dentin that was treated with 7% maleic acid and 17% EDTA, a result similar to that obtained by Ballal et al. (2010).

Based on these results, it is evident that 7% maleic acid affected the microhardness least and was quite effective for removing smear layers at all the three zones that were studied. Moreover, MTAD and 10% maleic acid were the most effective in removing the smear layer from all zones but considerably reduced the microhardness. Thus, 7% maleic acid can be considered to be effective in the removal of the smear layer compared with other irrigants.

Furthermore, studies are required on the effectiveness of 7% maleic acid for the removal of smear layer and its effect on dentin's organic content, with a greater number of samples and a longer observation period. However, with the current evidence, the use of 7% maleic acid is recommended for the removal of the smear layer to increase the sealing ability of obturating materials, thereby increasing the overall success of endodontic therapy.[36]

CONCLUSION

Based on this study's limitations, 7% maleic acid was as effective as MTAD for removing the smear layer from the apical third region without considerably affecting dentin's microhardness.

CONFLICT OF INTEREST

The authors do not have any financial interest in the companies whose materials are mentioned in this article.

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