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Effect of Triton, HEDP and EDTA Chelating Irrigant's Protocol on Micro-Hardness of Root Canal Dentine: An *In Vitro* Study

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Abstract

Aim of the study: To assess and compare the effectiveness of continuous chelating irrigant's protocol (Triton, 1-hydroxyethylidene-1,1-bisphosphonate (HEDP) with 5.25% sodium hypochlorite, and with conventional chelation protocol 5.25% NaOCl followed by 17% ethylenediamine tetra-acetic acid (EDTA)) on root canal dentine micro-hardness.

Material and method: In this study, forty mandibular premolars extracted for orthodontic purposes were utilized, the teeth were then categorized into four groups based on the type of irrigation solution, Group A; Triton, Group B; HEDP, Group C; EDTA, Group D; Distilled water (control). The crowns were removed to produce root length of 14 mm, ProTaper Next files up to size 40/0.06 were used for teeth preparation, then the roots separated into two parts. The micro-hardness was evaluated at three thirds (coronal, middle, and apical) for each half, 3 N load was used for 15sec. Statistical analysis of the data was performed using one-way ANOVA, followed by the Tukey post hoc test. The level of significance was $p \leq 0.05$.

Results: Triton had the least effect on micro-hardness, while EDTA had the highest effect. All chelating agents decrease micro-hardness with no significant difference among them. However, they different significantly from the control group ($p \leq 0.05$) at coronal third.

Results: All chelating agents reduced micro-hardness in the three thirds of root canal (coronal, middle, and apical). Among them, Triton and HEDP offer an effective balance between preservation dentine strength and effective cleaning, making them suitable option for endodontists focused on maintaining root strength during treatment.

Keywords: Chelating irrigants, Triton, HEDP, EDTA, micro-hardness, root dentine

Introduction

Root canal treatment is based on the preparation of the root canal system chemomechanically, combining instrumentation with the powerful irrigation (Elika et al., 2021). Irrigation is important, not only at shaping process but also it aids the removal of dentinal debris and bacteria through its rinsing action (Ali et al., 2022). Additionally, it avoids the accumulation of debris within the apical area and decrease the risk of spreading of periapical infection (Gomes et al., 2023).

The standard protocol involves the sequential application of sodium hypochlorite (NaOCl) at a concentration 0.5%-5.25%, followed by 17% ethylenediamine tetra-acetic acid (EDTA) and a final rinse with distilled water (Dutner et al., 2012; Mostafa et al., 2020). Although this protocol has proven its efficacy for smear layer removal, studies have shown that this protocol have the potential to change the chemical structure of dentine in particular the content of calcium in hydroxyapatite. Such changes may affect other properties of the tooth such as its micro-hardness and pemeability (Sayin et al., 2009, Augusto et al., 2022). The evaluation of micro-hardness provides information about changes in physical and chemical properties of dentine, including alteration in mineral contents, micro-hardness, and the modulus of elasticity (Unnikrishnan et al., 2019). Each capsule of HEDP (Medcem GmbH, Weinfelden, Switzerland) containing 0.9 of etidronic acid which should be added to 10mL of fresh NaOCl. The resulting solution maintain its activity within 1 hour (Zehnder, 2019).

Triton, recently introduced. It consists of two parts with varying composition: Part A contains chelating agent, pH modifiers, surfactants and stabilizers, part B includes 8% NaOCl and pH modifiers. These components are mixed using an automix technique to produce the final solution, which contains 4% NaOCl. This solution enables the simultaneous dissolution of both organic and inorganic tissues. The non-NaOCl ingredients in Triton actively break down dentinal debris, minimizing the buffering action required when organic debris encounters the reduced NaOCl concentration. By eliminating the need for multiple irrigants and sterile water rinses, Triton streamlines the procedure and reduces chair side time (Wakas Oraim and Al-Zaka, 2024).

According to best authors' knowledge, there are controversies about the effect of continuous chelation using HEDP and Triton on micro-hardness of root dentine. Therefore, the aim of the present study was to assess and compare the effectiveness of continuous chelating irrigant's protocol (Triton, HEDP with 5.25% NaOCl, and with conventional chelation protocol 5.25% NaOCl followed by 17% EDTA) on root canal dentine micro-hardness. The null hypothesis was that different irrigation protocols would not affect dentine micro-hardness.

Materials and methods

This study was received ethical approval from the Research Ethics Committee at the College of Dentistry, Mustansiriyah University (Approval Number. MUOPR 29, Date: 1/5/2023). This study utilized forty lower premolars extracted for orthodontic reasons. The selection of teeth were based on radiographic and clinical examination, and these criteria were teeth with single root and canals, absence of caries, and mature apex. The teeth were kept in distilled water to prevent dehydration. The crowns were sectioned at cemento-enamel junction using diamond disc to get standardize root length of 14mm, the working length were determined 1mm short of the apex. The teeth were then divided according to type of irrigation into four groups (n=10 for each group).

Group A: Triton (Brasseler; Savannah, USA): canals were irrigated with 8 mL of Triton during instrumentation; 6 mL was used as the final irrigation for 2 min.

Group B: Dual rinse (HEDP) (Medcem GmbH, Weinfelden, Switzerland): Immediately before root canal treatment, 10 mL of 5.25% NaOCl was added with 9% HEDP (one capsule), and mixed for 1-2 min according to manufacturer's instructions, until homogenous solution was obtained. The canals were then irrigated with 8 mL during instrumentation; 6 mL was used as the final rinse for 2 min.

Group C: 17% EDTA (Cerkamed, Poland): During instrumentation, 8 mL of 5.25% NaOCl was applied, then 5 mL of distilled water was used for rinsing. 6 mL of 17% EDTA was applied as final rinse for 2 min.

Group D: (Distilled water): During instrumentation, distilled water 8mL was utilized. 6 mL of distilled water were then utilized as the final irrigation for 2 min.

All teeth were finally rinsed with 5mL of distilled water to remove any remnants of chemical irrigants. Root canal irrigation was performed by a 5mL syringe (Heze Yinuo Medical Industry China) and a 30 Gauge endodontic needle (SinaliDent, China) which inserted 2mm away from the apex. ProTaper Next files (Dentsply Maillefer) up to size 40/0.06 at speed 300 and torque 2Ncm were used for preparation of root canals.

Following completing the endodontic treatment, two grooves were created longitudinally on both the lingual and buccal aspects of the roots utilizing a diamond disc operated at low speed. This procedural step was crucial to facilitate the subsequent separation of the roots into two distinct segments using a chisel. Each half-root was subsequently embedded horizontally in an acrylic block, enabling a secure and consistent environment for measurement. The halves was divided into three thirds: coronal, middle, and apical thirds, using an indelible marker for precise identification. For micro-hardness measurement at each third, a Vickers microhardness tester was

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used, applying a 3 N load for a duration of 15 seconds (Arul et al., 2021).

Statistical analysis

For statistical analysis evaluation, SPSS version 22.0 (IBM, Armonk, NY, USA) was used. To determine the normality of the data, Shapiro-Wilk test was used. Differences among the groups were analyzed using one-way ANOVA, followed by Tukey's post-hoc test for multiple pairwise comparisons. Statistical significance was defined as $p \leq 0.05$.

Results

To analyze the normality of the data, The Shapiro-Wilk test was performed, the result was the data were normally distributed. The mean and standard deviation (±SD) of Vickers micro-hardness values (for all samples in three thirds (coronal, middle and apical) are recorded in Table 1.

Table1. ANOVA statistical analysis of micro-hardness values for three thirds (coronal, middle, and apical).

Groups	Thirds									F	p value
	Coronal			Middle			Apical				
	Range	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD		
Group A	42.7 -69.6	57.147	10.259	40.0-63.5	51.899	8.423	33.9-93.8	64.341	23.330	1.625	0.216
Group B	31.8-67.0	50.083	11.793	20.4-69.2	46.125	15.127	41.3-87.0	66.794	19.647	4.789	**0.017**
Group C	32.2-64.5	48.324	11.993	25.7-62.4	39.103	12.057	37.6-66.0	52.123	10.072	3.443	**0.047**
Group D	46.7-100.8	73.664	18.675	34.9-88.4	59.680	16.927	50.9-93.8	81.343	12.221	4.612	**0.019**
F		7.246			4.165			4.869			
P value		**0.001**			**0.014**			**0.008**			

SD: Standard deviation

The highest mean of micro-hardness was found in control group at three thirds. In experimental groups Triton had the highest mean of micro-hardness, while the lowest mean showed by EDTA group. In coronal third, there was no significant difference ($p > 0.05$) among experimental groups. However, all experimental groups were significantly difference ($p \leq 0.05$) with distilled water. In middle and apical thirds the only significant difference ($p \leq 0.05$) was found between EDTA and distilled water as shown in Table 2. The comparison among thirds in each group, in Triton group no significant difference ($p > 0.05$) was found among thirds. In other three thirds, there was significant difference ($p \leq 0.05$) between middle- apical thirds, while no

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significant difference (p > 0.05) was found between (coronal-middle and coronal-apical thirds) as seen in Table 3.

Table 2. Tukey's post-hoc test of surface micro-hardness among groups.

Thirds	Groups		MD	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
Coronal	A	B	7.06400	0.653	-9.2839	23.4119
		C	8.82300	0.475	-7.5249	25.1709
		D	-16.51700	**0.047**	-32.8649	-.1691
	B	C	1.75900	0.991	-14.5889	18.1069
		D	-23.58100	**0.002**	-39.9289	-7.2331
	C	D	-25.34000	**0.001**	-41.6879	-8.9921
Middle	A	B	5.77400	0.776	-10.5155	22.0635
		C	12.79600	0.168	-3.4935	29.0855
		D	-7.78100	0.577	-24.0705	8.5085
	B	C	7.02200	0.655	-9.2675	23.3115
		D	-13.55500	0.132	-29.8445	2.7345
	C	D	-20.57700	**0.009**	-36.8665	-4.2875
Apical	A	B	-2.45300	0.989	-23.1495	18.2435
		C	12.21800	0.397	-8.4785	32.9145
		D	-17.00200	0.139	-37.6985	3.6945
	B	C	14.67100	0.242	-6.0255	35.3675
		D	-14.54900	0.249	-35.2455	6.1475
	C	D	-29.22000	**0.003**	-49.9165	-8.5235

MD: Mean difference.

Table 3. Tukey's post-hoc test of surface micro-hardness among thirds.

Groups	Thirds		MD	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
B	Coronal	Middle	3.95800	0.843	-13.6194	21.5354
		Apical	-16.71100	0.065	-34.2884	.8664
	Middle	Apical	-20.66900	**0.019**	-38.2464	-3.0916
C	Coronal	Middle	9.22100	0.186	-3.4319	21.8739
		Apical	-3.79900	0.740	-16.4519	8.8539
	Middle	Apical	-13.02000	**0.043**	-25.6729	-.3671
D	Coronal	Middle	13.98400	0.149	-3.9484	31.9164
		Apical	-7.67900	0.545	-25.6114	10.2534
	Middle	Apical	-21.66300	**0.016**	-39.5954	-3.7306

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Discussion

The chemical composition of dentine can modify by endodontic irrigants which affect on its organic and inorganic components, and this result in a reduction in micro-hardness of dentine and make tooth more susceptible to fracture. Thus, selection of irrigants require careful consideration to ensure maximum effectiveness with minimal impact on root canal dentine (Elika et al., 2021). The penetration depth and the effects of therapeutic materials used on dentine during endodontic treatment rely on number and diameter of dentinal tubules (Ghisi et al., 2014). In the present study, micro-hardness of root canal dentine was measured in three thirds of dentine. The micro-hardness is influenced by the presence of hydroxyapatite in the intertubular substance and extent of mineralization (Saha et al., 2017). The micro-hardness are important clinically, as they offer a measure of the amount of force needed for the cohesive bond failure within dentine (Sim et al., 2001) and also reflect increase or decrease in minerals of dentine (Zaparolli et al., 2012). The micro-hardness alteration after root canal therapy may increase the risk of tooth fracture (Reeh et al., 1989). For micro-hardness measurement, either Vickers indenter or Knoop indenter micro-hardness tests can be used (Dineshkumar et al., 2012). However, Oliveira et al., 2007 have found that Vickers micro-hardness test is suitable in the analysis of dentine surface alteration exposed to chemical agents. Therefore, to assess the superficial layer of dentine of the root canal lumen, Vickers micro-hardness test was used in this study.

This study showed that distilled water had a minimal effect affect the micro-hardness, which is in agreement with earlier studies that used distilled water as a negative control (Saghiri et al., 2009; Elika et al., 2021; Sahebi et al., 2023). Among experimental groups, Triton had the least reduction on micro-hardness and this may be attributed to the NaOCl concentration in Triton which is (4%) after automixing. While EDTA caused the highest effect and this agree with many previous studies (Taneja et al., 2014; Baldasso et al., 2017) that showed the adverse effect of EDTA on dentine micro-hardness. HEDP also decrease micro-hardness but its effect lower than EDTA and this finding agree with previous study by Ulusoy et al., in 2020. The decrease in micro-hardness in all experimental groups may be attributed to NaOCl. Several studies have demonstrated a considerable reduction in dentine micro-hardness after treatment by 2.5% NaOCl

(Ari et al., 2004, Sahebi et al., 2020, Philip et al., 2021). This reduction is attributed to the low surface tension of NaOCl, which allows it to diffuse into the dentine and penetrate narrow, elongated dentinal tubules through capillary action (Sahebi et al., 2023). Additionally, NaOCl aids in this reduction by breaking down long peptide chains and chlorinating the terminal groups of proteins (Philip et al., 2021). Both Triton and HEDP showed decrease in microhardness. However, this decrease was less than that in EDTA group. The reason for that may be due to when a soft chelating irrigation protocol is applied, there is greater intertubular dentine space available for hybridization (De-Deus et al., 2008). EDTA has potent chelating action, causes softening on the calcified portion of dentine. The reduction in micro-hardness is most likely due to the saturation of its cation-binding sites with calcium ions from the root dentine (Zaparolli et al., 2012).

The null hypotheses which stated that micro-hardness was not influenced by different irrigation protocols was rejected. The limitation of this study was that micro-hardness didn't measure before chemo-mechanical preparation.

Conclusions: All chelating agents reduced micro-hardness in the three thirds of root canal (coronal, middle, and apical). Among them, Triton and HEDP offer an effective balance between preservation of strength of root canal dentine and effective cleaning, making them suitable option for endodontists focused on maintaining root strength during treatment.

Supplementary Material

None.

Author Contributions

Tamara Nabhan Al Tai: data curation, writing-original draft preparation. Iman Mohammed Al-Zaka: Conceptualization, methodology, writing-review and editing.

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Data Availability Statement

Data are available from the authors upon reasonable request.

Conflict of interest

The authors reported that they have no conflicts of interest.

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