RESEARCH PAPER

An Ex Vivo Comparative Study Impact of Irrigation Protocols on Root Dentin Microhardness

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ABSTRACT

Purpose: The objective of this ex vivo experiment was to compare and assess the effect of standard and new irrigation protocols - Ethylenediaminetetraacetic acid (EDTA), Sodium Hypochlorite (NaOCl), Nanochitosan (NC) - in terms of root dentin microhardness.

Methods: A total of 100 human premolars with a single root were instrumented and randomly divided into five groups (n=20) including Group 1 (5.25% NaOCl + 17% EDTA), Group 2 (5.25% NaOCl + 0.2% Nanochitosan), Group 3 (1% Heated NaOCl + 17% EDTA), Group 4 (1% Heated NaOCl + 0.2% Nanochitosan), and Group 5 (Normal Saline). A Vickers microhardness tester with a load of 100g lasting 10s was used to assess the coronal, middle, and apical dentin microhardness at baseline and after the irrigation plans. Paired t-tests and ANOVA were used in statistical analysis.

Results: After the irrigation, the EDTA-containing procedures (Groups 1 and 3) had a very high significant reduction of microhardness at each of the root thirds ($p < .001$ in Group 1). The two groups also reported the lowest final post-treatment microhardness values that were significantly lower than those reported by the other groups in the coronal and middle thirds ($p < 0.01$). Surprisingly, the nanochitosan protocols (Groups 2 and 4) exhibited a much superior preservation of the dentin hardness, with Group 4 having the lowest loss (6.1 Vickers Hardness Number VHN), similar to that of the control (saline).

Conclusion: Within the limitations of this ex vivo study, the root dentin microhardness of the irrigation regimens that include 17 percent EDTA significantly decreases. The protocols that used nanochitosan as the chelating agent (Groups 2 and 4) were far less harmful, which means that they can be used as the viable alternatives to maintaining the integrity of dentin.

Keywords: Dentin Microhardness, Endodontics, EDTA, Nanochitosan, Sodium Hypochlorite.

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INTRODUCTION

Effective endodontic treatment is the total elimination of microbial organisms, residual pulpal, and smear layer contents in the complex system of the root-canal (Duncan et al., 2023). This is achieved through thorough disinfection, optimal shaping and complete obturation within the root canal systems thereby contributing to ensuring long-term survival of the treated tooth (Duncan et al., 2023; Fransson & Dawson, 2023). Mechanical instrumentation alone is not often sufficient because of anatomical complications such as lateral canals, fins, isthmuses and dentinal tubules that can interfere with the contacts of the instruments to all the canal walls (Siqueira et al., 2018; Versiani et al., 2023; Versiani et al., 2021). There is empirical evidence that mechanical preparation can leave a canal wall untouched 35 to 50% (Liu et al., 2022; Razumova et al., 2020). Chemical irrigation has therefore proved to be an essential complement to instrumentation, not a mere complement to instrumentation but a main process in removing tissues, disinfecting them and removing the smear-layer (Srivastava, 2024; Tomson et al., 2025). Endodontic success is, therefore,

centered on high-quality irrigation (Valizadeh et al., 2024). Lack of efficient irrigant activity, residual microbial load, tissue remnant and smear-layer debris compromise penetration of sealer, which interferes with microorganism and decreases the cohesion of filling agents (Buurma & Buurma, 2020). Empirical evidence also shows that the stronger levels of irrigants provide better microbial elimination and tissue dissolution along with the risks of dentin structural degradation (Baruwa et al., 2022; Wong et al., 2021).

Sodium hypochlorite (NaOCl) is primarily used as the major irrigant in modern practice. It is effective due to its strong bactericidal effect and ability to solubilize organic tissue warranting its widespread usage (Gulabivala & Ng, 2023; Qutieshat et al., 2023; Susila et al., 2023). It has been recorded that concentrations of 0.5 to 5.25 percent NaOCl result in a high level of antimicrobial effects in infected root canals, and the higher concentrations of NaOCl destroy a broader range of organisms (Barakat et al., 2024; Konadu et al., 2024). In other studies, it has been demonstrated that 5.25 NaOCl had a significant negative effect on the fracture strength of root dentine reducing it to 114.58 ± 26.74 MPa compared to the 172.10 ± 30.13 Mpa, which is a concentration-dependent adverse effect on the integrity of dentin (de Almeida Rodrigues et al., 2019; Xu et al., 2022). In this way, high concentrations like 5.25% and more are most effective but, at the same time, have high cytotoxicity and can severely impair the mechanical characteristics of dentin (Bapat et al., 2021; Gomes et al., 2001; Jiang et al., 2017; Luz et al., 2019; Severing et al., 2019). To the contrary, lower concentrations like 1% and lower are less effective but safer in microbial destruction and tissue dissolution (Gomes et al., 2001; Luz et al., 2019; Severing et al., 2019).

Simultaneously, a chelating agent, ethylenediaminetetraacetic acid (EDTA) is the standard final rinse (Guo et al., 2018; Unnikrishnan et al., 2019). This is due to the fact that NaOCl alone cannot be used to eliminate the inorganic part of the smear layer. The chelating effect of EDTA removes calcium ions in the dentin, and it removes the smear-layer and opens dentinal tubules to enhance adaptation of sealer (Ozel et al., 2024; Unnikrishnan et al., 2019). Nevertheless, EDTA is associated with complications as it causes root-dentine erosion on long-term exposure, and dentin microhardness, and in the event of consecutive use with NaOCl, it can also trigger an unfavorable combined effect that further undermines the dentin microstructure (Baruwa et al., 2022; Ozel et al., 2024). Following that within the consecutive usage, NaOCl and EDTA provides a better smear-layer elimination at the cost of increased dentin erosion and reduced mechanical strength (Tartari et al., 2017; Turk et al., 2015). The main paradox, then, is that the best chemical irrigation guidelines, especially ones that involve using high-level concentration of NaOCl and EDTA, are also the most harmful to dentin (Guo et al., 2018; Turk et al., 2015). This brings about a trade-off of better cleaning or maintenance of dentin integrity.

Therefore, a new irrigation protocol will be required to achieve an effective elimination of microbial tissue and to maintain dentin microstructure (Baruwa et al., 2022; Ozel et al., 2024; Wong et al., 2021). The first issue is sodium hypochlorite (NaOCl), namely the ability to define how low-concentration NaOCl can be as effective as high-concentration NaOCl without causing dentin damage. The intracanal heating of NaOCl to increase its tissue-dissolving and antimicrobial effects is one of the solutions postulated, yet its effect on the dentin microstructure has not been explored in depth. The second challenge is directed at EDTA where there is a question of whether it is possible to replace it with a safer chelating agent. Nano-chitosan (chitosan nanoparticles) has potential due to its biocompatibility, chelating and antibiofilm characteristics. Although its impact on removed smear-layer and on microhardness have been researched, its ability as an EDTA alternative has not been clearly determined. It is also worth noting that no ex-vivo research has investigated the interaction of heated low-concentration NaOCl and a nano-chitosan rinse on dentin microstructure.

This ex vivo study tried to determine the effect of different irrigation regimens on microhardness of dentin in root-canals. In particular, the research aimed to examine the effect of these protocols on dentin microhardness (Vickers hardness) and also to assess the structural alterations, including erosion, in the dentin microstructure, which may occur. This study aims to compare the effects of five irrigation protocols on dentin microhardness. The main outcome measure was dentin microhardness (Vickers hardness) change that occurred at the start and the end of the treatment. A special focus was made on Protocol 4 that used a hot low-concentration NaOCl mixed with nano-chitosan as a final rinse. This new strategy has been developed with a specific aim of

increasing cleaning effectiveness with minimum possible harm to the integrity of dentin.

METHODS

This ex vivo experiment is designed to compare and assess the effect of standard and new irrigation regimens in terms of root dentin microhardness.

Inclusion Criteria

The samples included were single-rooted, single-canal and intact human mandibular pre-molar of morphological similarity, no prior endodontic therapy, a root length of at least 14 mm and an apical diameter of at least #20.

Exclusion Criteria

Teeth with root caries, internal resorption, cracks or curved and obliterated canals were excluded.

Sample Selection and Preparation

This study was approved by the Research and Ethics Committee at College of Dentistry, Qassim University (No: 24-92-09)One hundred single rooted and single canal intact human mandible premolars extracted due to reasons not associated with this study were identified. To verify one patent canal radiographs (mesial and distal) were done. The crowns were cut 2mm above the (cementoenamel junction CEJ) to act as an irrigant reservoir. Root lengths were normalized to 15.0 $\pm$ 1 mm with a double faced diamond disc under water cooling. Longitudinal sectioning of specimens of each group was done. Buccal and lingual aspects were incised, and a chisel and mallet were used to bisect the roots. The acrylic resin was poured on one side of each specimen . A Vickers Diamond Microhardness Tester (Shimadzu HMV-G31DT Vickers Micro Hardness Tester) was used with a 100-gram load in ten seconds at 100µm and 300µm above the canal lumen.

Experimental Groups and Irrigation Protocol

The samples were randomly divided into five different groups of 20 units each. The sealing of the apical foramina was done using composite resin, and a layer of nail varnish was used to seal the outside surfaces. The Protaper Gold Rotary System was used to prepare all root canals to a size of size F3 (30.09).

1. **Group 1 (5.25% NaOCl + EDTA):** Each instrumentation step would be followed by the application of 2ml of 5.25% NaOCl (60s) and an additional 5ml (60s) after the completion of each step. Two minutes of terminal flush with 3ml of 17% EDTA was applied.
2. **Group 2 (5.25% NaOCl + Nanochitosan):** The irrigation sequence was the same as in Group 1 except that the final rinse was 3ml of 0.2% nanochitosan, which was applied at two minutes.
3. **Group 3 (Heated 1% NaOCl + EDTA):** 2 ml of 1% NaOCl was added between files and 60s were allowed. This was followed by the activation of 5.0 ml 1% NaOCl in the internal activation of the System-B Heat Source (180^0C) with an X-fine tip, turned on 8s and left to rest 10s. This process was carried out 10 times with the irrigant being refreshed in between. The 17% EDTA last flush of 3ml was done in two minutes.
4. **Group 4 (Heated 1% NaOCl + Nanochitosan):** Group 4 repeated the internal heating protocol of Group 3, except that the 3ml/2min irrigation was followed. The terminal flush was 0.2% nanochitosan.
5. **Group 5 (Control):** 2ml normal saline 60s between instrumentation periods and post-prep wash of 5ml.

Outcome Assessment

Post irrigation measurement of microhardness were recorded as previously described

Statistical Analysis

The numerical data processing was done using (Statistical Package for the Social Sciences SPSS) (version 26.0). The values of dentin microhardness were calculated using mean and standard deviation (Standard deviation SD). A paired-samples t-test was used to compare microhardness before and after irrigation in each of the experimental cohorts (Table 1). In order to compare the microhardness of all the five independent groups at baseline and post-irrigation, one-way Analysis of Variance (ANOVA) was used (Table 2). The analysis was significant at p =0.05.

RESULTS

There were no failures in preparation of all 100 specimens in accordance with the laid down protocols. The microhardness analysis was done in two phases where intra-group analysis was used to determine the effect of each protocol (Table 1) and inter-group analysis to determine relative differences between the protocols (Table 2).

The comparison of microhardness values before and after irrigation for each specific group is presented in Table 1.

Table 1: Comparison of Dentin Microhardness Values (Mean ± SD) at Coronal, Middle, and Apical Thirds Before and After Irrigation with Different Solutions

Group			Coronal (Values)			Middle (Values)			Apical (Values)		
			Mean	Standard Deviation	Pvalue	Mean	Standard Deviation	Pvalue	Mean	Standard Deviation	Pvalue
5.25% NaOCl (3 ml 17% EDTA (2 min))	Irrigation	Pre	62.8	7.7	<.001	64.1	5.2	<.001	58.3	7.1	<.001
		Post	43.4	4.2		43.1	5.4		42.9	2.7	
5.25% NaOCl (3 ml 0.2% Nanochitosan (2 min))	Irrigation	Pre	52.8	14.3	0.25	59.6	10.3	0.15	59.3	7.3	0.02
		Post	47.0	12.1		52.4	10.6		49.5	5.7	
NaOCl 1% + Internal Heating (3 ml 17% EDTA (2 min))	Irrigation	Pre	59.4	8.7	<.001	58.5	7.6	0.01	53.0	9.5	0.03
		Post	42.1	3.5		43.5	6.5		41.7	1.5	
NaOCl 1% + Internal Heating(3 ml 0.2% Nanochitosan (2 min))	Irrigation	Pre	65.3	6.4	0.04	68.1	5.6	0.11	56.8	17.1	0.30
		Post	57.8	5.4		62.1	5.1		51.2	15.6	
Normal Saline	Irrigation	Pre	62.1	5.0	0.23	64.0	4.4	0.23	55.6	11.4	0.41
		Post	60.0	3.8		62.1	3.4		53.9	11.7	

Table 1. Data are presented as Mean ± Standard Deviation (SD) in Vickers Hardness Number (VHN). The p-value represents the statistical significance of the change from Pre- to Post-irrigation (paired t-test) for each group in each root third.

The intra-group changes showed that protocols with EDTA had a high level of microhardness reduction. Group 1 (5.25 percent NaOCl + EDTA) showed a very significant reduction in all root thirds (Coronal, Middle, Apical; $p < 0.001$). All thirds also showed significant decreases in Group 3 (1 of NaOCl + EDTA) (Coronal, $p < 0.001$; Middle, $p=0.01$, Apical, $p= 0.03$). On the other hand, protocols containing no EDTA had a better preservation of dentin: Group 4 (1% NaOCl+ Internal Heating) showed only a small, but significant, coronal loss ($p=0.04$). The significant reduction was only on the apical third ($p=0.02$) with Group 2(5.25% NaOCl + Nanochitosan). The saline control (Group 5) showed no statistically significant changes in the root of any third.

A between-group analysis was performed to compare the microhardness of all five groups at baseline and after the intervention. The results are shown in Table 2.

Table 2: Pre- and Post-Treatment Dentin Microhardness (Mean ± SD) at Coronal, Middle, and Apical Thirds Following Different Irrigants

Irrigation			Coronal (Values)			Middle (Values)			Apical (Values)		
			Mean	Standard Deviation	Pvalue	Mean	Standard Deviation	Pvalue	Mean	Standard Deviation	Pvalue
Pre	**Group**	5.25% NaOCl (3 ml 17% EDTA (2 min))	62.8	7.7	0.26	64.1	5.2	0.23	58.3	7.1	0.91
		5.25% NaOCl (3 ml 0.2% Nanochitosan (2 min))	52.8	14.3		59.6	10.3		59.3	7.3	
		1% NaOCl (3 ml 17% EDTA (2 min))	59.4	8.7		58.5	7.6		53.0	9.5	
		1% NaOCl + Internal Heating (6 ml, System-B 180°C × 10 cycles)	65.3	6.4		68.1	5.6		56.8	17.1	
		Normal Saline	62.1	5.0		64.0	4.4		55.6	11.4	
Post	**Group**	5.25% NaOCl (3 ml 17% EDTA (2 min))	43.4	4.2	<0.01	43.1	5.4	<0.01	42.9	2.7	0.19
		5.25% NaOCl (3 ml 0.2% Nanochitosan (2 min))	47.0	12.1		52.4	10.6		49.5	5.7	
		1% NaOCl (3 ml 17% EDTA (2 min))	42.1	3.5		43.5	6.5		41.7	1.5	
		1% NaOCl + Internal Heating (6 ml, System-B 180°C × 10 cycles)	57.8	5.4		62.1	5.1		51.2	15.6	
		Normal Saline	60.0	3.8		62.1	3.4		53.9	11.7	

Table 2. Data are presented as Mean ± Standard Deviation (SD) in VHN. The p-value represents the statistical significance of the difference between the five experimental groups at each time point (ANOVA).

Inter-group comparison did not show any baseline microhardness differences between the five groups (Coronal p=0.260, Middle p=0.230, Apical p=0.910) which showed the completion of the randomization. Post-irrigation showed significant variations in the coronal and middle thirds ($p<0.01$), which highlights the difference in the effect of the irrigation regimens on the dentin hardness. The EDTA containing groups (Group 1: 43.4 VHN, Group 3: 42.1 VHN) had the lowest final hardness and the control (Group 5: 60.0 VHN) and the heated group (Group 4: 57.8 VHN) had better preservation in the coronal region. The mean of the middle third showed similar pattern, but no significant variance was seen in the apical division ($p = 0.190$).

The absolute reduction in microhardness (Pre-irrigation VHN − Post-irrigation VHN) for each group is visualized by root third in Figures 1-3.

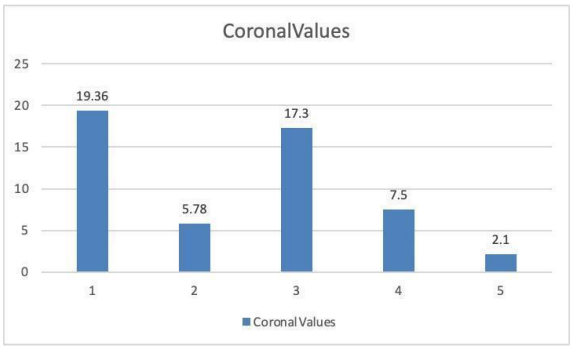

Figure 1. Mean Absolute Reduction in VHN (Pre-Post) in the Coronal Third

Figure 1: The chart shows the magnitude of microhardness loss in the coronal third. Groups: 1 (5.25% NaOCl+EDTA), 2 (5.25% NaOCl+NC), 3 (1% NaOCl+EDTA), 4 (1% NaOCl+NC), 5 (Saline).

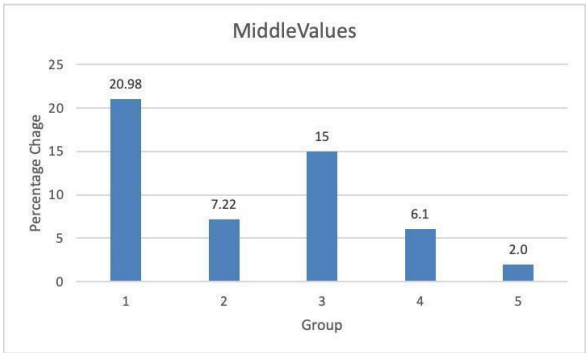

Figure 2. Mean Absolute Reduction in VHN (Pre-Post) in the Middle Third

Figure 2: The chart shows the magnitude of microhardness loss in the middle third. Groups: 1 (5.25% NaOCl+EDTA), 2 (5.25% NaOCl+Nano), 3 (1% NaOCl+EDTA), 4 (1% NaOCl+Heating), 5 (Saline).

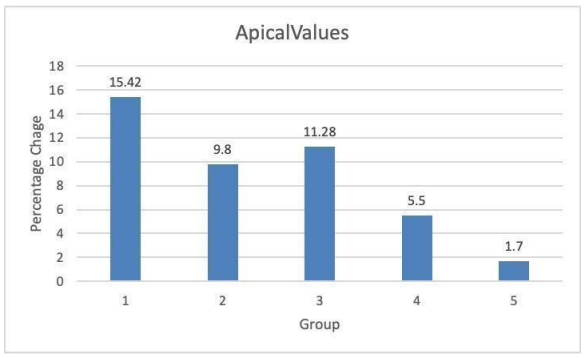

Figure 3. Mean Absolute Reduction in VHN (Pre-Post) in the Apical Third

Figure 3: The chart shows the magnitude of microhardness loss in the apical third. Groups: 1 (5.25% NaOCl+EDTA), 2 (5.25% NaOCl+Nano), 3 (1% NaOCl+EDTA), 4 (1% NaOCl+Heating), 5 (Saline).

Complete changes in microhardness, which are in pre-irrigation VHN versus post irrigation VHN of each group on the root thirds, are depicted in Figures 1-3. These values are consistent with the fact that the highest loss of dentin hardness was observed in EDTA containing protocols: Group 1 exhibited losses of 19.36 (Coronal), 20.98 (Middle), and 15.42 (Apical) VHN; Group 3 exhibited losses of 17.3 (Coronal) and 15.0 (Middle) VHN. Conversely, the lack of EDTA was more effective in preserving microhardness: Group 4 and Group 2 showed significantly lower reductions whereas saline control (Group 5) did not show significant reductions.

DISCUSSION

This ex-vivo study assessed the comparative impact of irrigation protocols on dentin hardness. The main findings of this study are that the irrigation regimens with a percentage content of 17% EDTA (Groups 1 and 3) provided a statistically significant and clinically significant decrease in the root dentin microhardness, independent of the sodium-hypochlorite concentration used. In comparison, the protocol that used 1% intracranal NaOCl with a nano-chitosan rinse (Group 4) and the protocol using 5.25% NaOCl with nano-chitosan final rinse (Group 2) maintained dentin microhardness significantly better (Table 2; Figures 1-3). Numerically, the middle-third Vickers hardness is lower here as EDTA is the dominant driver of hardness loss, while alternatives and procedural modifications can moderate that effect.

The harmful impact of EDTA (Groups 1 and 3)

The fact that 17% EDTA was observed in this research to have the largest decreases in Vickers hardness is consistent with a large and consistent literature of laboratory results. Several in-vitro studies have established significant and time- and concentration-dependent microhardness reductions under the influence of 17% EDTA or other aggressive chelators (Hazar & Hazar, 2025; Kaul et al., 2023; Khabadze et al., 2025; Martinez-Andrade et al., 2018; Muana et al., 2021; Unnikrishnan et al., 2019). These reports show that the VHN changes have a range which is similar to the current findings (average reported changes are between about 10 and 40 VHN based on exposure time, tooth area and test depth) (Kaul et al., 2023; Muana et al., 2021; Unnikrishnan et al., 2019). They usually explain the process by mineral chelation and expansion of intertubular spaces which reduce the dentin mineral fraction and effective load-carrying cross-section. More recent comparison articles also highlight that EDTA causes a higher erosion and hardness loss than other acids or chelators when used under similar circumstances (Kaul et al., 2023; Muana et al., 2021; Wahyuniwati et al., 2016). The current middle-third decreases thus aligning with these reports and supporting the fact that the chelating effect of EDTA is the intervening factor behind the increased decrease in hardness in Groups 1 and 3 (Wahyuniwati et al., 2016).

NaOCl concentration impact on dentin hardness

One of the main results can be made in the process of the comparison of Group 1 (5.25% Unheated NaOCl + EDTA) and Group 3 (1% Heated NaOCl + EDTA). As it was shown in our results, Group 1 showed a significantly higher decrease in microhardness (around 20.98 1/VHN in the middle of the third) compared to Group 3 (around 15.0 1/VHN). This finding indicates the 5.25% content of NaOCl as a greater source of dentin harm as compared to the intracanal heated 1% NaOCl. The result is consistent with the rest of the literature that reports that high-concentration NaOCl more severely destroys the organic matrix, therefore contributing to the further mechanical weakening caused by chelating agents (Barakat et al., 2024; Kafantari et al., 2019; Massoud et al., 2017). Some studies have also shown that heating may also favor collagen breakdown, but in the presence of EDTA, concentration is the most destructive factor (Kafantari et al., 2019; Massoud et al., 2017). Therefore, the small loss in hardness, which is experienced in Group 1, albeit slightly bigger, is mechanistically valid and supports a number of previous studies. It is important to note that differences in the literature have been experienced in terms of exposure period and temperature; therefore, the temporal differentiation of the effect of the concentrations may vary between researches (Kafantari et al., 2019; Massoud et al., 2017).

Nanochitosan's (Group 2) protective effect

The promising findings were achieved using Group 4, which used a 1% intracanal heated NaOCl and a 0.2% nanochitosan rinse. As shown in the findings, the use of nano -chitosan (Group 2) instead of the final rinse considerably prevented the loss of hardness (middle third, 7.22 VHN) compared to 17 per cent EDTA groups. This is harmonious with an emerging literature of indications that chitosan and its nanoparticles forms are less aggressive chelators, and in certain studies at least, they cause low levels of mineral loss and small amounts of microhardness decay in contrast with EDTA (Ratih et al., 2020; Rosalia et al., 2020; Veeraiyan et al., 2023). These experiments reiterate the more conservative chelating behavior of chitosan and how the latter may be used to remove the smear layer through adsorption and mild chelation as compared to the demineralizing effect of the more violent approach (Ratih et al., 2020; Rosalia et al., 2020; Ilhan et al., 2024; Veeraiyan et al., 2023). In-vitro studies yielded a meta-analysis that found chitosan-treated dentin preservation to have better VHN than EDTA with similar differences in magnitude, as is seen in the present study (single-digit to low-teen VHN, depending on procedure and concentration) (Alves et al., 2024). Although other authors have reported that heating NaOCl can increase its disinfection efficiency, the first evidence of this has been obtained by our study that indicated that this could be done without mechanical loss, on the condition that an aggressive chelator is not used. The protocol therefore achieves a clear distinction between the intended disinfection (that is a result of heating) and the undesired demineralization (that is a result of EDTA).

The effect of intracanal heating of 1% NaOCl (Group 4)

Group 4 that used a combination of intracanal heating of 1% NaOCl with no potent chelator showed slight loss of hardness ≈ 6.10 VHN (middle third) but statistically the same as in the saline in this dataset.

The fact is significant since it implies that heating a low-concentration NaOCl can be used to increase its antimicrobial and tissue-dissolving qualities (as reported elsewhere) without collateral tissue damage in comparison with EDTA or high-concentration NaOCl. Previous studies assessing heated NaOCl have revealed enhanced effects of heating NaOCl in situ on organic dissolution and smear-layer (del Carpio-Perochena et al., 2015; Ribeiro et al., 2022; Morago et al., 2016), but there is a conflicting body of literature on the effect of heating NaOCl on dentin mechanical properties (Barakat et al., 2024; Iandolo et al., 2018; Tartari et al., 2021). It has been reported that heating may increase collagen denaturation rate and decrease viscoelasticity with increase in temperature, and other intracanal heating procedures have reported better cleaning with minimal mechanical loss when temperatures and contact times are regulated (Al-Nasrawi, 2019; Barakat et al., 2024; De Santis et al., 2022; Kafantari et al., 2019). The current findings indicate that the regulated intracanal heating of low concentration of NaOCl can produce improved functional performance without any significant loss of hardness, an observation that differs with the ones that heated a higher concentration or used a longer duration of exposure. This means that there are interactions between temperature, concentration and exposure time, and it can be optimized that low-concentration NaOCl can be heated to maintain dentin mechanical integrity (Agarwal et al., 2025; Shruthi, 2023).

Clinical implications

Clinically, the results suggest that EDTA based final rinses, although capable of doing a good smear-layer removal, have a quantifiable and potentially clinically important mechanical cost in the form of dentin hardness. Intracanal application of a non-aggressive chelator like nano -chitosan challenged with heated 1% NaOCl seems to retain hardness but does not miss out on any of the organic and inorganic components of the smear layer. Clinicians ought to be aware that a high concentration of 5.25% NaOCl is more harmful to the microhardness than the 180 C intracanal heating utilized in this case. Group 4 (1 per cent Heated NaOCl + Nanochitosan) provides a good way to develop the protocol and balances the assumed efficacy of heating with the confirmed mechanical safety of a non-EDTA chelator. Therefore, protocols that avoid long-term contact with EDTA and favor alternative strategies, including charged chelators, nanoparticles, and procedural modifications, including heating low - NaOCl intracanal, now provide an attractive justification of clinical trials. Such tests need to reconsider the classic gold standard and instead favor the approach that pays attention to dentin maintenance, particularly in structurally undermined teeth.

Limitations & Future Scope

This study has certain limitations that should be acknowledged including how the study as an ex vivo lacks the ability to fully replicate the complexities of in vivo buffering processes, pulpal residue effects, periodontal ligament effects, or the long-term processes of mechanical remodelling that are clinically realized. This particular enquiry used one type of tooth morphology (mandibular premolars) and molar anatomy and dentin variability potentially would produce divergent results. Microhardness is an instantaneous mechanical surrogate that does not include long-term fracture resistance or fatigue life, or bond strength of obturation materials, which are critical to clinical translation. Future studies ought, therefore, to include standardized in vivo models or well-developed clinical trials, prolong follow-up periods to determine the effects of mechanical fatigue and conduct parametric studies by controlling sodium hypochlorite temperature, concentration, and nano-chitosan formulations to develop a safe and effective clinical protocol. The results of this paper support additional investigation of the role of heated low-concentration NaOCl in association with nano-chitosan as solutions that can balance the disinfection and preservation of dentin until this data is available.

CONCLUSION

Based on the findings of this study, the type of irrigants and its selection with activation technique has significantly impacted the microhardness of the root dentin. The use of the irrigation protocols of 17% EDTA when combined with 5.25% NaOCl, showed greatest reduction of hardness of dentin preservation and highlights the promise of nano-biopolymers as less aggressive preservatives. It is important to note that on the other hand, intracanal heating of 1% NaOCl and the 0.2% nano-chitosan procedures led to very limited hardness loss, which was statistically equivalent to that seen with saline, hence it has the potential to act as a safe but effective irrigant modality. Such results imply that the traditional EDTA-based irrigation paradigm can be reconsidered.

Especially due to the favor of protocols that ensure the protection of dentin without sacrificing the effectiveness of antimicrobials.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

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