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Original Research

Assessment of the Microhardness of Root Canal Dentin by Different Irrigating Solutions

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ABSTRACT:
Background: Endodontic irrigation plays a critical role in root canal disinfection, but its impact on dentin microhardness remains a clinical concern. Loss of dentin hardness may compromise the long-term structural integrity of endodontically treated teeth. **Aim:** This study aimed to evaluate and compare the effects of various commonly used irrigating solutions on the microhardness of root canal dentin. **Materials and Methods:** Forty extracted human premolars were sectioned and grouped into five categories: distilled water (control), 5.25% sodium hypochlorite (NaOCl), 17% EDTA, 2% chlorhexidine (CHX), and 10% citric acid. Each sample was immersed in the respective solution for 5 minutes. Pre- and post-treatment Vickers microhardness values were measured. Data were analyzed using paired t-tests and ANOVA. **Results:** EDTA and citric acid significantly reduced dentin microhardness ($p < 0.001$), followed by NaOCl ($p < 0.01$). CHX had minimal effect, and distilled water showed negligible change. Intergroup comparisons confirmed statistically significant differences. **Conclusion:** Chelating agents such as EDTA and citric acid cause the most significant reduction in dentin microhardness. CHX appears to preserve dentin integrity better. Clinicians should carefully select irrigants to balance disinfection efficacy with preservation of dentin structure.
Keywords: Dentin microhardness; Endodontic irrigants; EDTA; Sodium hypochlorite; Chlorhexidine

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INTRODUCTION
Successful root canal treatment relies not only on mechanical debridement but also on the chemical dissolution of organic and inorganic debris using endodontic irrigants. During root canal preparation, the smear layer—comprising dentin debris, pulp tissue remnants, and microorganisms—forms on the canal walls and acts as a barrier to disinfectants and sealers [1]. Therefore, effective irrigation protocols are essential to achieve thorough canal decontamination and dentin conditioning.
Among the numerous properties expected from an ideal irrigant—antimicrobial efficacy, tissue dissolution capacity, smear layer removal, and biocompatibility—its impact on dentin microstructure is often underemphasized [2]. One critical aspect of dentin integrity is microhardness, which reflects the mineral content and mechanical resistance of dentin to functional stresses and subsequent restorative procedures [3]. Alterations in dentin microhardness can significantly influence its bonding characteristics, resistance to fracture, and long-term treatment prognosis [4].
Sodium hypochlorite (NaOCl) remains the most widely used irrigant due to its tissue-dissolving and antimicrobial properties. However, its potential to degrade organic components in dentin and reduce hardness has been documented [5]. Similarly, chelating agents like ethylenediaminetetraacetic acid (EDTA) and citric acid effectively remove the smear layer but can decalcify dentin, leading to structural weakening [6,7]. In contrast, chlorhexidine (CHX) is

known for its substantivity and lower dentin erosiveness, although it lacks tissue-dissolving capacity [8]. Novel agents such as herbal irrigants and nanoparticle-based solutions have also emerged, claiming superior biocompatibility with minimal impact on dentin integrity [9,10].

The effect of these irrigating solutions on dentin microhardness has been investigated in several in vitro studies with varying outcomes, depending on concentration, exposure time, and testing method. As preserving dentin structure is vital for post-endodontic restoration, understanding how different irrigants affect microhardness is essential.

This study aims to assess and compare the effects of commonly used root canal irrigating solutions on the microhardness of root canal dentin, using standardized testing techniques. By elucidating the relative demineralizing potential of each irrigant, the findings may help clinicians select appropriate solutions that balance disinfection with dentin preservation.

MATERIALS AND METHODS

Sample Selection and Preparation

Forty freshly extracted single-rooted human mandibular premolars with intact roots and no signs of caries, cracks, or resorption were collected. The teeth were thoroughly cleaned using an ultrasonic scaler to remove soft tissue remnants and stored in 0.1% thymol solution until use. The crowns were sectioned at the cementoenamel junction using a low-speed diamond disc under water cooling to obtain standardized root segments.

Each root was then split longitudinally using a chisel and mallet to expose the canal dentin. From each root, a 3 mm thick mid-root dentin slice was prepared using a precision saw under continuous water irrigation. The slices were polished with silicon carbide papers (400–600 grit) to produce a uniform, flat surface for microhardness testing.

Grouping and Irrigating Solutions

The specimens were randomly divided into five groups (n=8 per group), each subjected to a different irrigating protocol:

Group I (Control): Distilled water
Group II: 5.25% Sodium hypochlorite (NaOCl)
Group III: 17% Ethylenediaminetetraacetic acid (EDTA)
Group IV: 2% Chlorhexidine gluconate (CHX)
Group V: 10% Citric acid

Each specimen was immersed in 5 mL of the respective solution for 5 minutes, simulating clinical exposure time. After treatment, specimens were rinsed with distilled water and stored in saline until testing.

Microhardness Testing

The Vickers microhardness test was employed to evaluate dentin surface hardness before and after irrigant exposure. A microhardness tester with a Vickers diamond indenter was used, applying a 200 g load for 15 seconds. Three indentations were made at equidistant points on the flat dentin surface, and the average value was recorded for each specimen. Post-treatment microhardness was calculated using the same method at nearby locations, avoiding overlap with prior indentations.

Statistical Analysis

The data were compiled using Microsoft Excel and analyzed with SPSS software version 24.0. Descriptive statistics were calculated as mean and standard deviation (SD) for each group. Intragroup comparisons (pre- and post-treatment) were performed using paired t-tests. Intergroup comparisons were analyzed using one-way ANOVA followed by Tukey’s post hoc test. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Baseline Microhardness Values (Pre-treatment)

All dentin samples exhibited comparable microhardness values before treatment. There was no statistically significant difference among the five groups ($p > 0.05$), confirming the homogeneity of the samples at baseline.**Table 1**

Post-treatment Microhardness Comparison

After application of irrigating solutions, significant differences were noted in dentin microhardness. The **greatest reduction** was observed in the **EDTA** group (mean VHN 45.4, $p < 0.0001$), followed by **Citric Acid** (mean VHN 47.2, $p < 0.0005$). **NaOCl** also significantly reduced dentin hardness (mean VHN 52.1, $p = 0.001$). In contrast, **CHX** (mean VHN 58.3, $p = 0.012$) and **Distilled Water** (mean VHN 60.9, $p = 0.37$) had minimal impact on microhardness. **Table 2**

Intergroup Post-treatment Comparisons

Statistical comparison using ANOVA and Tukey's post hoc test revealed that **EDTA and Citric Acid** groups had significantly lower microhardness than NaOCl and CHX groups ($p < 0.005$). No significant difference was found between EDTA and Citric Acid ($p = 0.186$). CHX maintained significantly higher hardness than EDTA and Citric Acid ($p < 0.01$), supporting its dentin-preserving properties.**Table 3**

Percentage Reduction in Microhardness

The **percentage decrease in microhardness** was highest in the EDTA group (26.9%), followed by Citric Acid (22.5%), NaOCl (14.3%), and CHX (4.7%). Distilled water showed minimal change (0.8%), confirming its neutral effect. **Table 4**

Table 1: Baseline Microhardness (Pre-treatment)

Group	Mean Microhardness (VHN)	Standard Deviation
Distilled Water (Control)	61.4	2.1
NaOCl 5.25%	60.8	2.5
EDTA 17%	62.1	2.2
CHX 2%	61.2	2.0
Citric Acid 10%	60.9	2.3

Table 2: Post-treatment Microhardness

Group	Mean Microhardness (VHN)	Standard Deviation	p-value (vs. baseline)
Distilled Water	60.9	1.8	0.37
NaOCl 5.25%	52.1	2.4	0.001
EDTA 17%	45.4	2.9	0.0001
CHX 2%	58.3	1.9	0.012
Citric Acid 10%	47.2	2.6	0.0005

Table 3: Intergroup Comparison (Post-treatment)

Group Comparison	p-value	Significance
NaOCl vs EDTA	0.003	Yes
NaOCl vs CHX	0.009	Yes
EDTA vs CHX	0.002	Yes
CHX vs Citric Acid	0.001	Yes
EDTA vs Citric Acid	0.186	No

Table 4: Percentage Reduction in Microhardness

Group	Baseline VHN	Post-treatment VHN	Reduction (%)
Distilled Water	61.4	60.9	0.8
NaOCl 5.25%	60.8	52.1	14.3
EDTA 17%	62.1	45.4	26.9
CHX 2%	61.2	58.3	4.7
Citric Acid 10%	60.9	47.2	22.5

DISCUSSION

The present study was designed to evaluate the effect of various commonly used endodontic irrigants on the microhardness of root canal dentin. Microhardness is directly related to the mineral content of dentin and serves as a proxy for its mechanical resilience, resistance to fracture, and long-term integrity following root canal therapy [11]. The results indicate that different irrigating agents significantly impact dentin hardness, with chelating agents producing the most pronounced reduction.

At baseline, all specimens displayed statistically similar dentin microhardness values, ensuring group comparability. However, post-treatment evaluation revealed a notable decline in microhardness values for samples exposed to NaOCl, EDTA, and Citric Acid. These findings align with previous research indicating that strong oxidizing and demineralizing agents adversely affect dentin integrity by altering its collagen matrix and inorganic content [12,13].

EDTA emerged as the most aggressive irrigant in terms of reducing dentin hardness, with a 26.9% decrease in VHN values. This is consistent with earlier studies that have shown EDTA's potent calcium-chelating action leads to significant mineral loss and surface erosion, especially when used in high concentrations or for prolonged durations [14]. The current study used a 17% solution for 5 minutes, which, while clinically common, proved sufficient to cause dentin weakening. Similar trends were observed with **Citric Acid**, which also acts by chelating calcium ions but has been reported to cause slightly more surface etching than EDTA [15].

Sodium hypochlorite (NaOCl), despite being primarily a tissue solvent and antimicrobial, also demonstrated a moderate but statistically significant reduction in microhardness (14.3%). This is likely due to its ability to degrade organic components, particularly collagen, within the dentinal matrix. NaOCl does not decalcify dentin directly but compromises the structural framework by denaturing proteins, thereby weakening the overall microstructure [16]. Its effect, however, was less severe than that of chelators like EDTA or Citric Acid.

Interestingly, **Chlorhexidine (CHX)** showed the least reduction in microhardness among the tested active agents (4.7%), indicating its relative biocompatibility with dentin. CHX lacks tissue-dissolving and chelating properties, which may explain its minimal impact on the mineral content of dentin. This makes CHX a potentially valuable agent in protocols where preservation of dentin hardness is prioritized, such as in cases requiring bonding of fiber posts or reinforcement strategies [17].

The **control group (distilled water)** showed negligible changes, validating that the observed reductions in other groups were due to the chemical effects of the irrigants. The findings also emphasize that while smear layer removal is a necessary step in endodontic disinfection, it must be balanced against the risk of weakening dentin, which could predispose the tooth to structural failure [18].

From a clinical standpoint, these results support the recommendation that **chelating agents should be used judiciously**, possibly as a final rinse and for a limited duration. Moreover, the sequential or alternating use of NaOCl and EDTA should be approached with caution, as studies have reported synergistic effects leading to greater structural compromise [19].

In terms of methodology, the **Vickers hardness test** was chosen for its reliability in detecting changes in hard tissues. While other studies have used Knoop or nanoindentation, the Vickers method provides reproducible results for comparative analysis of surface microhardness. However, it should be noted that in vitro testing may not completely replicate intraoral conditions, and factors such as saliva, temperature, and dentinal fluid may moderate the effects observed in this study [20].

Additionally, the duration of irrigant exposure, while standardized for experimental consistency, may not reflect actual clinical protocols where the contact time is shorter or interrupted. This suggests that the clinical impact on dentin microhardness may be less dramatic but nonetheless relevant, especially in the context of minimally invasive endodontics.

CONCLUSION

This study demonstrated that commonly used endodontic irrigants have varying effects on the microhardness of root canal dentin. Among the tested solutions, **EDTA** and **Citric Acid** caused the most significant reduction, highlighting their strong demineralizing action. **Sodium hypochlorite** also showed a moderate decrease in dentin hardness, while **chlorhexidine** had the least impact, indicating better preservation of dentin structure. These findings emphasize the importance of carefully selecting irrigants based on clinical objectives, particularly when long-term structural integrity is crucial. Clinicians should consider limiting the exposure time and concentration of chelating agents to reduce potential weakening of dentin. Further in vivo studies are warranted to validate these in vitro observations and refine irrigation protocols.

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