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Effect of Endodontic Irrigants on Root Dentin Microhardness: A Systematic Review

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Abstract: ***Removal of the organic and inorganic components of the smear layer in the course of the endodontic treatment is achieved by the use of different endodontic irrigants. Despite their favorable disinfection abilities, they are reported to alter the biomechanical properties of root canal dentin. The aim of this article is to critically review published in vitro studies comparing effects of different irrigation regimes on dentinal microhardness and to identify, synthesize, and analyse the data available over the five-year period from March 2015 to March 2020. The literature search was done on PubMed - MEDLINE, The Cochrane Library and Science Direct database, then the articles were assessed by two independent reviewers. A total of thirteen papers out of 134 titles met the eligibility criteria. All studies reported reduction of root dentin microhardness after treatment with various endodontic solutions. EDTA reduced dentin microhardness to a greater extent in comparison with other solutions regardless of the application time and the total amount of the chelating agent. Photon-Induced Photoacoustic Streaming proved to be a safe method for additional activation of disinfection solutions.***

Keywords: endodontic irrigants; root dentin; microhardness.

1. Introduction

Chemical disinfection during the endodontic treatment aims to clean the complex root canal system remaining insufficiently cleaned and shaped by instruments could not be directly shaped by instruments. The ideal irrigation solution should provide lubrication, debridement, dissolution of the organic and inorganic ingredients of the smear layer as well as antimicrobial effect towards various pathogenic microorganisms and their toxins. There is no evidence to date for a s single irrigant able to fulfil all these requirements [1, 2].

Although adequate smear layer removal can only be achieved by the simultaneous use of shaping instruments and different irrigating solutions, the latter can exhibit detrimental alterations in the chemical composition of dentin [3, 4, 5]. The change of Ca/P ratio affects the original proportion of organic to inorganic components, thus modifying dentin's microhardness, solubility, permeability and surface roughness. According to some researchers these effects are time- and concentration-dependent [6, 7, 8].

Microhardness testing is a comprehensive, non-destructive and easily performed method utilized for investigation of the fine scale changes in the hardness of the non-homogenous dentin structure [9, 10, 11]. It is determined as material's resistance to local deformation which is measured on the basis of the permanent surface deformation that remains after removal of a given load [11]. The effect of microhardness on the overall outcome of the endodontic treatment has yet to be evaluated clinically. Nevertheless, *in vitro* tests that determine the hardness profile of root dentin can provide indirect evidence of any change of its mineral composition [12, 13]. Such changes could influence the adhesive properties of the root dentin surface and decrease the root strength and its resistance to fracture [7, 14].

Reports in literature are inconsistent regarding the optimal application time and concentration of the endodontic irrigants necessary for achieving a thorough smear layer removal without affecting the biomechanical properties of root dentin [3]. Recently, there is a paradigm shift in the utilization of the most commonly used disinfecting solutions. Several studies compare the effects of sodium hypochlorite and ethylenediaminetetraacetic acid with newly suggested alternative irrigants (phytic acid, etidronic acid, herbal products) in terms of their ability to impair the dentinal matrix [15, 16, 17].

Thus, considering the available literature, the aim of this systematic review was to investigate the effect of recently used endodontic irrigants on the root dentin microhardness.

2. Material and methods

2.1. Eligibility criteria

The review process was performed on articles assessing the *in vitro* effect of various regimens of conventional and/or alternative irrigation solutions on specimens prepared from extracted, fully formed, non-endodontically treated human teeth without any root canal caries, fillings, resorption, cracks or fractures.

2.2. Exclusion criteria

Articles investigating the change of other parameters of the root dentin (such as smear layer removal, surface roughness, etc.) along with its microhardness were not included in the systematic review.

2.3. Literature search and data extraction

A thorough literature search was done on PubMed -

MEDLINE, The Cochrane Library and Science Direct database. A total of four combinations of the following keywords: root dentin, microhardness, irrigation, protocol, solutions were used to gather the required literature using appropriate filters.

The literature search was performed for in vitro studies investigating the effect of different endodontic irrigants published during the period from 31st March 2015 to 31st March 2020. The language was restricted to English. A similar search strategy was also applied to manual searches, including journals and reference lists.

2.4. Screening and selection

The relevance of each paper to the criteria was determined by two independent reviewers based on its title and/or abstract. Case reports, letters and reviews were not included in the search. If the keywords were present in the title and/or the abstract, the papers were selected for further full-text reading. Papers without abstracts but with titles suggesting that they were related to the objectives of this review were also selected to screen the full text for eligibility. After selection, full-text papers were read in details by the two reviewers. Those papers that fulfilled all of the selection criteria were processed for data extraction. The reviewers hand searched the reference lists of all selected studies for additional relevant articles. Any disagreements between the two reviewers were resolved by discussion.

From the collection of papers that met the eligibility criteria, data were extracted with respect to the change of the root dentin microhardness after the effect of various endodontic solutions used in different regimens in terms of their application time and concentration.

3. Results

In the PubMed – MEDLINE database 106 articles were obtained with the combination of the keywords. The overall number of papers found through Cochrane Library, Science Direct and manual search was: 6, 10 and 8, respectively. After removing duplicates for searches and identifying relevant studies 134 unique works were chosen for further systematic review. A total number of 13 articles were finally chosen for quantitative synthesis (Fig.1). Table 1 shows characteristics of the studies reviewed about the effect of endodontic irrigants on root dentin microhardness.

4. Discussion

All of the tested irrigation regimens used in the reviewed articles reduced root dentin microhardness [2, 6, 7, 9, 11, 16-22]. These findings are in confirmation of previous studies where endodontic disinfection solutions are reported to cause alterations of the mechanical properties of radicular dentin [3, 23-27].

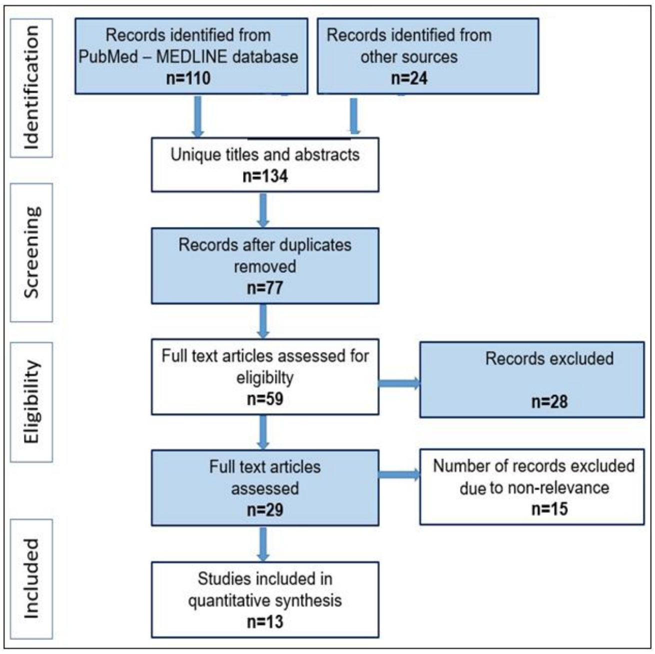

Figure 1. The PRISMA diagram, depicting the flow of information through the different phases of a systematic review.

Almost all of the studies preferred longitudinal over the transverse sectioning of the intact roots into discs [2, 6, 7, 9, 11, 15, 16-22]. Earlier investigation of *Cruz-Filho et al.* revealed that cutting the root into buccal and lingual half represent the clinical situation more accurately [14]. Half of the research teams preserved the integrity of the root canal wall by keeping the morphology of the dentinal surface close to its original state [2, 6, 7, 9, 15, 22]. Thus, they were able to evaluate the sole effect of the disinfection solutions on the root dentin microhardness. Other research teams provided a plane for measurement that has been previously shaped either manually or by engine-driven rotary instruments [11, 16-21]. Despite resembling the clinical situation better, it might be speculated that the reduction of microhardness of dentin by this approach is due to the cumulative action of the instrumentation and the irrigation solutions. Another important aspect to be considered in the comparison of dentin hardness values is whether the test was carried out in the same sample and region before and after the irrigation treatment [28]. In an attempt to overcome the morphological differences between the teeth several authors tested the baseline microhardness value of each sample prior the influence of the irrigants. Thus, each specimen served as its own control [2, 9, 17, 18, 20, 22]. In another study (*Akbulut et al.*, 2019) one half of the previously sectioned roots was tested, while the other remained as a control group [21]. In order to overcome the bias of the evaluation, other investigators advocated a control group, where all the samples were immersed in distilled water [19, 6, 7, 11, 16] since it is not considered as a variable which might affect the dentinal surface microstructure [23, 29]. Moreover, *Duvvi et al.* and *Rapgay et al.* utilized both initial measurement and control group in their experimental design [2, 17].

All of the measurements were done by Vicker's hardness indenter [2, 6, 7, 9, 11, 15-22]. The usage of just one type of indentation for all surface treatments along with the accurate readings of this methodology make it convenient for registration of surface changes of hard dental tissues treated with chemical agents [9, 13, 21]. Nevertheless, there is a lack of consensus in the literature regarding the number of indentations, distance from the lumen, load and duration of the application of the indenter. More than half of the studies performed the testing in the mid-level of the root dentin [2, 6, 7, 9, 19, 21, 22]. The choice of this specific area might be due to the non-homogenous structure of dentin. The tubule density increases from cervical to apical dentin, resulting in an inverse correlation between dentin microhardness and tubule number [2, 22]. Based on the assumptions of *Pashley et al.* (1985) that dentin hardness values decrease as the indentations are made close to the root canal lumen [30], some authors chose the area halfway between the center of the canal lumen and the peripheral cementum as the dentin structure there is more uniform [6, 9, 22].

Reports from research data have been inconsistent in regard to the time, concentration and sequence of the commonly used irrigants for achieving proper disinfection without causing deleterious changes of the mechanical properties of dentin tissue. The studies included in the current review article predominantly analyzed the microhardness change after the single use of an endodontic irrigant [2, 7, 9, 11, 15, 16, 17, 19]. Their findings confirmed the ability of various concentrations of NaOCl and chelating agents to reduce dentinal microhardness to a different extent due to the softening of the root canal walls and the erosion of the tubules. The greatest alteration of mechanical properties of dentin was registered under the demineralizing action of 17% EDTA [7, 9, 11, 16, 17, 21]. In order to simulate the clinical conditions, various authors suggest the 5-minute duration of the endodontic irrigation [2, 6, 7, 16, 17, 20] which is in accordance with previous studies [14, 24, 25, 31]. *Nikhil et al.* advocated a 3-minute action of the irrigation solutions [15], whereas other authors opted for a shorter duration [18, 21]. Their results confirmed the findings of *De-Deus et al*. (2006) who claimed that the single use of 17% EDTA produced the greatest decrease in microhardness from reference state to 3 minutes [24]. In an attempt to overcome the deficiencies of these solutions, some authors suggest alternative agents such as 7% maleic acid [18], 1% phytic acid [15], 0.2% chitosan [9, 15, 16], 6%MCJ (Morinda citrifolia juice) [9], etidronic acid [16], 5% and 10%CaOCl2 [2], Tea tree oil [17], 5% Tamarindus indica [17] and 5% Green tee extract [17].

Additional activation of the disinfection irrigants might lower dentin microhardness compared to that of the non-activated ones. *Arslan et al.* assessed the effect of 808-nm diode laser agitation of EDTA. Their findings demonstrated that the highest reduction of hardness values was observed in the group with the longest duration of the laser action [19]. Results from a recent study of *Quteifani et al*. showed that activation of irrigants with Er: YAG laser could minimize their adverse effects on dentine microhardness. The discrepancy between these two studies might be due to the higher penetration ability of diode lasers [22]. Another novel approach facilitating the disinfection capacity of the endodontic irrigants is their Photon-Induced Photoacoustic Streaming (PIPS) activation. PIPS uses an erbium-doped yttrium aluminum garnet (Er:YAG) laser producing a photoacoustic shock wave. Although this technique enables powerful streaming of the irrigant throughout the root canal system, *Akbulut et al.* registered no additional alteration in dentin microhardness after its utilization [21]. No information was found on the influence of the ultrasonic activation of the irrigants in the reviewed research data.

5. Limitation

Although, the major databases were used for the literature search, papers that are not listed in these sources might have been omitted. The current review includes articles published in English language, which may have excluded potentially valuable evidence. Extensive literature can be found related to the most commonly used endodontic irrigants such as NaOCl, EDTA, chlorhexidine. Few studies focused on methodologies for additional activation of the solutions.

6. Conclusion

All irrigation regimens decreased the mechanical properties of root canal dentin. EDTA reduced dentin microhardness to greater extent in comparison with other solutions regardless of the application time and the total amount of the chelating agent. Photon-Induced Photoacoustic Streaming proved to be a safe method for additional activation of disinfection solutions.

7. Conflict of interest

The authors declare no conflict of interest.

8. Acknowledgements

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Table 1: Summary of characteristics of the studies reviewed about the effect of endodontic irrigants on root dentin microhardness. (EDTA – ethylenediamine tetraacetic acid; CA – citric acid; MA-maleic acid; AA– acetic acid; SC – sodium citrate; RCT– root canal treatment; US– ultrasonic agitation; LA – laser activation; NS normal saline; MCJ– Morinda citrifolia

juice; * statistcal significance)

Author/year	Type and sample size	Specimen preparation	Irrigation protocol	Microhardness measurement	Microhardness reduction
1. ***Kara Tuncer*** et al., 2015 [18]	40 maxillary canines	• Decoronated at CEJ • RCT - **ProTaper**, • Irrigation: 2 ml 2.5% NaOCl/ 30 after each file • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing • **Baseline microhardness**	**Groups (n=20):** Method: **Immersion of 5ml/1 min** for each solution in the regimen: • 17% EDTA+2.5% NaOCl • 17% EDTA+2% CHX • QMix2in1 • 7% MA **Interim and final rinse**: deionized and distilled water	**Vickers diamond indenter** • Indentations: **3** • Level: **coronal, middle and apical third** • Distance: **100µm** from the lumen • Load/time: **200g/20s**	• **Apical region**: MA* > QMix = 17% EDTA + 2% CHX* > 17% EDTA + 2.5% NaOCl • **Middle region:** MA* > QMix = 17% EDTA + 2% CHX = 17% EDTA + 2.5% NaOCl • **Coronal region:** MA* > QMix = 17% EDTA + 2% CHX = 17% EDTA + 2.5% NaOCl
2. ***Arslan*** et al., 2015 [19]	42 maxillary anterior teeth	• Decoronated at CEJ • RCT – **barbed broach K files #40** • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing • **Baseline microhardness**	**Groups (n=12):** Method: **Irrigation** • Negative control (15ml distilled water) • 5 ml 17 % EDTA • (1 ml 17 % EDTA+US/60s) + 2ml 17%EDTA/50s • (1 ml 17 % EDTA+**LA/10s**) + 4ml 17%EDTA/110s • (1 ml 17 % EDTA+**LA/20s**) + 3ml 17%EDTA/100s • (1 ml 17 % EDTA+**LA/30s**) + 2ml 17%EDTA/90s • (1 ml 17 % EDTA+**LA/40s**) + 1ml 17%EDTA/80s • **Final rinse**: 5ml 5%NaOCL/120s+ 5ml distilled water	**Vickers hardness tester** • Indentations: **3** • Level: **middle third** • Distance: **N/A** • Load/time: **15g/15s**	• All regimens decreased dentin microhardness. • **Greatest reduction**: (1 ml 17 % EDTA+**LA/40s**) + 1ml 17%EDTA/80s
3. ***Bakr*** et al., 2016 [7]	56 maxillary second premolars	• Decoronated at CEJ • Roots – sectioned **longitudinally** • Embedment: **acrylic resin**	**Groups (n=16):** Method: **Immersion of 5ml/5 min** for each solution: • NS • 5% EDTA • 17% EDTA • 2.5% NaOCl • 5% NaOCl • 0.2% CHX • 2% CHX	**Vickers hardness tester** • Indentations: **N/A** • Level: **middle third** Distance: **300 µm** from the lumen • Load/time: **500g/10s**	• **Greatest reduction**: 17%EDTA* • 5% EDTA*> NS, 0.2%CHX, 2% CHX • 5.25% NaOCl*> NS, 0.2%CHX, 2% CHX • EDTA*>NaOCl
4. ***Saleh*** et al., 2016 [6]	25 incisors	• Decoronated at CEJ • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing	**Groups (n=10):** Method: **Immersion** • Distilled water (control) • 5.25%NaOCl/10min+17%EDTA/1min • 5.25%NaOCl/10min+17%EDTA/5min • 5.25%NaOCl/20min+17%EDTA/1min • 5.25%NaOCl/20min+17%EDTA/5min • **Interim and final rinse**: 10ml distilled water	**Vickers hardness tester** • Indentations: **N/A** • Level: **middle third** Distance: **halfway from the outer surface** • Load/time: **300g/20s**	• All irrigation protocols reduced dentin microhardness significantly. • **Greatest reduction:** 5.25%NaOCl/20min+17%EDTA/5min
5. ***Nikhil*** et al., 2016 [15]	15 canines	• Decoronated at CEJ • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing	**Groups (n= 10):** Method: **Immersion + microbrush agitation** • 50 µL of 1% phytic acid/3 min • 50 µL of 17% EDTA /3 min • 50 µL of 0.2% chitosan/3 min • **Final rinse**: distilled water	**Vickers diamond indenter** • Indentations: **3** • Level: **coronal, middle and apical third** • Distance: **500µm** from the lumen • Load/time: **200g/10s**	• All the chelators reduced the dentin microhardness at all the levels • **Greatest reduction:** EDTA • EDTA*> phytic acid= chitosan
6. ***Massoud*** et al., 2017 [20]	40 mandibular premolars	• Decoronated at CEJ • RCT – **MAF: K-file#30** • Irrigation: 2 ml distilled water after each file • Roots – split **longitudinally** • Embedment: **acrylic resin** • Polishing • **Baseline microhardness**	**Groups (n=10):** Method: **Immersion of 10ml/5 min** for each solution: • 2.5%NaOCl • 17%EDTA+2.5%NaOCl • 2.5%NaOCl+2%CHX • 2.5%NaOCl+distilled water+2%CHX	**Vickers hardness tester** • Indentations: **N/A** • Level: **coronal, middle and apical third** Distance: **200µm** from the lumen • Load/time: **25g/10s**	• All irrigation protocols reduced dentin microhardness significantly. • **Greatest reduction**:17%EDTA+2.5%NaOCl • 17%EDTA+2.5%NaOCl*> 2.5%NaOCl+distilled water+2%CHX = 2.5%NaOCl+2%CHX*> 2.5%NaOCl • The coronal third of the root canal is the most affected third by irrigation solution.
7. ***Saha*** et al., 2017 [9]	80 premolars	• Decoronated at CEJ • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing	**Groups (n=20):** Method: **Immersion for 15 min** for each solution: • 3%NaOCl • 17%EDTA	**Vickers hardness tester** • Indentations: **3** • Level: **middle third** Distance: **halfway** between the centre of	• 17% EDTA = 0.2% Chitosan > 3% NaOCl =6% MCJ • 17% EDTA*> NaOCl =6% MCJ • 0.2% Chitosan*> NaOCl =6% MCJ

Author/year	Type and sample size	Specimen preparation	Irrigation protocol	Microhardness measurement	Microhardness reduction
7. ***Saha*** et al., 2017 [9]		• **Baseline microhardness**	• 0.2%Chitosan • 6%MCJ **Final rinse**: distilled water	the canal lumen and the peripheral cementum • Load/time: **300g/20s**	
8. ***Raghavendra*** et al., 2018 [16]	47 single-rooted teeth	• Decoronated at CEJ • RCT – **ProTaper-F3** • Irrigation: 2 ml 3% NaOCl/ 30s after each file • Final irrigation: : 20 ml 3% NaOCl • Roots – cleaved **longitudinally** • Embedment: **acrylic resin** • Polishing	**Groups (n=15 teeth):** Method: **Micropipette for 5 min** for each solution: • Control teeth (n=2) • Etidronic acid • 17%EDTA • 0.2%Chitosan **Final rinse**: saline	**Vickers hardness tester** • Indentations: **3** • Level: **1000, 1200, 1400µ** from the orifice • Distance: **N/A** • Load/time: **100g/15s**	• EDTA=Etidronic acid=Chitosan
9. ***Duvvi*** et al., 2018 [2]	75 mandibular premolars	• Decoronated at CEJ • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing • **Baseline microhardness**	**Groups (n=15):** Method: **Immersion of 5 min/5ml** for each solution: • Saline (control) • 2.5%NaOCl • 5%NaOCl • 5%$CaOCl_2$ • 10%$CaOCl_2$ **Final rinse**: saline	**Vickers hardness tester** • Indentations: **3** • Level: **middle third** • Distance: **300µm** from the lumen • Load/time: **300g/20s**	• All tested solutions decreased microhardness significantly. • 10%$CaOCl_2$ > 5%NaOCl > 5%$CaOCl_2$ >2.5%NaOCl • 10%$CaOCl_2$ =5%NaOCl
10. ***Rapgay*** et al., 2018 [17]	60 premolars	• Decoronated at CEJ • RCT – **MAF: K-file#40** • Irrigation: distilled water after each file • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • **Baseline microhardness**	**Groups (n=10):** Method: **Immersion for 5 min** • Saline (control) • Qmix • Tea tree oil • 5% *Tamarindus indica* • 5% Green tee extract • 17% EDTA **Final rinse**: 30ml saline	**Vickers diamond indenter** • Indentations: **3** • Level: **coronal, middle and apical third** • Distance: **500µm** from the lumen • Load/time: **200g/15s**	• EDTA> Qmix> *Tamarindus indica* • EDTA*> *Tamarindus indica* • Green tee= Tea tree=Saline

Author/year	Type and sample size	Specimen preparation	Irrigation protocol	Microhardness measurement	Microhardness reduction
11. ***Abbas*** et al., 2018 [11]	24 distal roots of mandibular molars	• Decoronated at CEJ • Embedment: **acrylic resin** • RCT – one shape rotary system • Irrigation: 1 ml distilled water after each file • Irrigation: 5 ml of each solution • Roots – sectioned **horizontally**	**Groups (n=6 roots):** Method: **Final irrigation with 5 ml of the solutions:** • 2.5% NaOCl • 17% EDTA • 40% CA • Distilled water	**Vickers hardness tester** • Indentations: **9** • Level: **coronal, middle and apical third** • Distance: **500µm** from the lumen • Load/time: **500g/20s**	• All solutions reduced the root dentin microhardness • EDTA>NaOCl>CA
12. ***Akbulut*** et al., 2019 [21]	72 mandibular premolars	• Decoronated at CEJ • RCT – **ProTaper Next-X3** • Irrigation: 1 ml distilled water after each file • Roots – sectioned **longitudinally** • Embedment: **acrylic resin** • Polishing	**Groups (n=12):** Method: **Needle irrigation with 6 ml/60s of the solutions:** • 2.5% NaOCl • 2% CHX • 17% EDTA • 2.5% NaOCl+PIPS • 2% CHX+PIPS • 17% EDTA+PIPS **Final rinse**: 2 ml distilled water	**Vickers hardness tester** • Indentations: **3** • Level: **middle third** • Distance: **100µm** from the lumen • Load/time: **300g/20s**	• **Greatest reduction**: EDTA • PIPS -no additional alteration in dentin microhardness
13. ***Quteifani*** et al., 2019 [22]	82 premolars	• Decoronated at CEJ • Roots – split **longitudinally** • Embedment: **acrylic resin** • **Baseline microhardness**	**Groups (n=12):** Method: • MTAD without LA • MTAD + LA • NaOCl + LA • NaOCl+17% EDTA +LA • NaOCl+MTAD +LA • NaOCl without LA • Distilled water (control) **Final rinse**: 2 ml distilled water	**Vickers hardness tester** • Indentations: **3** • Level: **middle third** • Distance: **halfway** from the outer surface • Load/time: **100g/20s**	• **Greatest reduction**: NaOCl without LA • Two-irrigants or no LA protocols > Single-irrigant or LA irrigation protocols