Obscure Fact ofEthylene Diamine TetraAceticAcid (EDTA)On Radicular Dentine:An in-vitro Study

Abstract

Background

Chelating agents are used to dissolve radicular dentin and eliminate the smear layer that develops after root canal preparation by mechanical means. By functioning as a barrier, the smear layer keeps irrigants from coming into direct touch with the dentin surface and dentinal tubules, cleaning them, and compromising the obturation's sealing qualities. Chelators like etidronic acid (1-hydroxyethane-1,1-diphosphonic acid [HEDP] pH 11) and phytic acid (PA) (inositol hexakisphosphate pH 1.3) have been suggested as EDTA substitutes recently. However, studies have shown that long-term usage of strong chelators, such as EDTA, may be detrimental to the biomechanical properties of root dentin, as seen by a decline in flexural strength and microhardness.

Aim and Objective

The objective of this *in vitro* study was to examine the impact of different endodontic chelating agents on the flexural strength and micro hardness of radicular dentin.

Materials and Method

Fourty dentin sticks were obtained from 10 single-rooted premolars and divided into four groups (*n* = 10). One stick from each tooth was assigned to one of the experimental groups and was soaked in one of the experimental chelating solutions for10 min, and 15 min in 17% ethylenediaminetetraacetic acid (EDTA), 2.5% phytic acid (PA), 18% etidronic acid, or saline (control group). Following the 10 min, and 15 min soak, the stick's flexural strength was evaluated using a 3-point loading test using the universal testing machine, and the surface microhardness was tested using a Vickers's microhardness tester.

Results:EDTA showed lower microhardness and flexure strength compared with the phytic acid and Etidronic acid.

Conclusion: Given the limits of this investigation, it can be stated that both 2.5% Phytic acid and 18% Etidronic acid chelators do not significantly degrade the surface and bulk mechanical properties of radicular dentin when compared to EDTA**.**

Keywords: Ethylene Diamine Tetra Acetic Acid,Endodontics,Chelating chemicals

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INTRODUCTION

Endodontics always requires chemomechanical debridement, which causes the creation of a smear layer on root canal surfaces.[1]The smear layer formed during mechanical instrumentation inhibits the effectiveness of irrigants, medicines, and root canal filling materials in dentinal tubules. [2]
EDTA (pH 8) is a reliable strong chelator that effectively removes smear layers.[3]Excessive usage of powerful chelators like EDTA might harm root dentin's biomechanical qualities, resulting in lower microhardness and flexural strength.[3]

To compensate for the deficiencies of these solutions, some authors suggest adding 7% maleic acid, 1% phytic acid, 0.2% chitosan, 6%MCJ (Morindacitrifolia juice), etidronic acid, 5% and 10% CaOCl2, tea tree oil, 5% Tamarindusindica, and 5% green tea extract .[8]

Alternatives to EDTA include phytic acid (PA) and etidronic acid (1-hydroxyethane-1,1-diphosphonic acid [HEBP] pH 11).[3]

Phytic acid (PA, inositol hexakisphosphate) is the primary phosphorus storage form found in plant seeds and bran, contributing to several cellular activities [4]

Etidronate, an aqueous solution containing 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), was introduced in 2005 as an endodontic chelating agent.[2]

METHODOLOGY

Dentin sticks preparation

Ten sound single- rooted premolars extracted for orthodontic reasons which were caries free was collected

Four radicular dentin sticks (1 mm × 1 mm × 12 mm) were extracted from each tooth root using a precision diamond disk with ample of water coolant.

Each tooth had one stick assigned to one of the four experimental irrigants.

Fig.1.Dentinsticks preparation

Irrigant preparation

Four irrigant solutions were used for this study

Group 1 (control): saline

Group 2: 17% EDTA

Group 3: 2.5% PA

Group 4: 18% etidronate

Solutions were used as manufacturer's instructions

Each stick was soaked in 1 mL of each individual irrigant solution in a sealed plastic tube before being placed in an ultrasonic vibrator for 10 and 15 minutes.

The stick was then cleaned with saline and immediately analyzed.

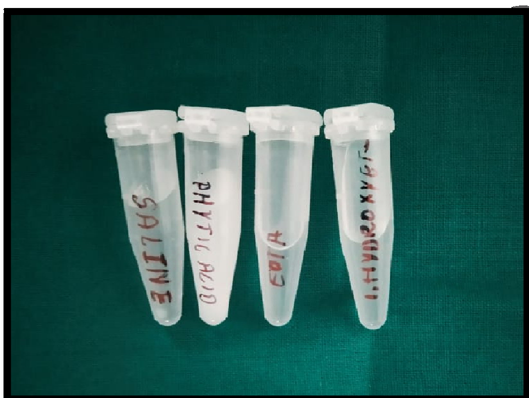


Fig. 2.Irrigant preparation

Flexural strength testing

"The flexural strength of the dentin sticks was measured using a universal testing machine. Each stick's dimensions were measured before testing to ensure precise calculation. The sticks were mounted on a metal support with a 1 mm diameter round support and a 10 mm support distance, then loaded with a round metal rod at a rate of 1 mm/min till failure" [4].

Microhardness testing

"The cracked dentin sticks were collected, and their Vickers hardness (HV) was determined using a microhardness tester. Three indentations were made on each stick, with a minimum gap of 1 mm between adjacent indentations. The load was applied smoothly and without impact by pushing the square diamond pyramid shape indenter into the test specimen with a 50 g (HV 0.1) weight for 10 seconds. After removing the load, the impression diagonals were measured three times using three different magnification settings and averaged, typically to the nearest 0.1-µm using a micrometer. The HV was computed using the formula HV = 1854.4 L/d2. The load (L) is measured in gf and the average diagonal (d) is in µm, resulting in hardness number units” [4].

Statistical analysis

The numerical data were expressed in terms of mean and standard deviation values.

Homogeneity assumption wasmet in the surface hardness data so they were analyzed using one-way ANOVA followed by Tukey’s *post hoc test.*

However, the assumption was violated in the flexuralstrength data so they were analyzed using Welch one-way ANOVA followed by Games-Howell *post hoc test*.

The significance level was set at *P < 0.001 within all tests*

Statistical analysis was performed with *R statistical analysis* software version 4.1.3 for Windows.

RESULTS AND DISCUSSION

Table 1. MEAN AND STANDARD DEVATION VALUES FOR FLEXURAL STRENGTH (MPa) AND SURFCAE MICROHARDNESS(VHN) AT 10 MINUTES

	Control	ETAD	Etidronate	phytic acid	P
Flexual Strength	197.91±2.92	71.26± 6.24	143.50 ±9.81	134.00 ±4.81	<0.001
Microhardness	44.48± 6.26	30.16 ±1.27	33.15 ±0.58	33.18 3±.26	<0.001

Table 2. MEAN AND STANDARD DEVATION VALUES FOR FLEXURAL STRENGTH (MPa) AND SURFCAE MICROHARDNESS(VHN) AT 15 MINUTES

	Control	ETAD	Etidronate	phytic acid	P
Flexual Stength	124.62 ±11.95	50.61 ±0.72	117.25 ±14.66	112.83 ±5.43	<0.001
Microhardness	38.38 ±0.28	27.77 ±0.71	31.52± 0.40	29.99 ±1.65	<0.001

Fig. 3. GRAPHICAL REPRESENTATION OF FLEXURAL STRENGTH AT 10 MINUTES AND15 MINUTES

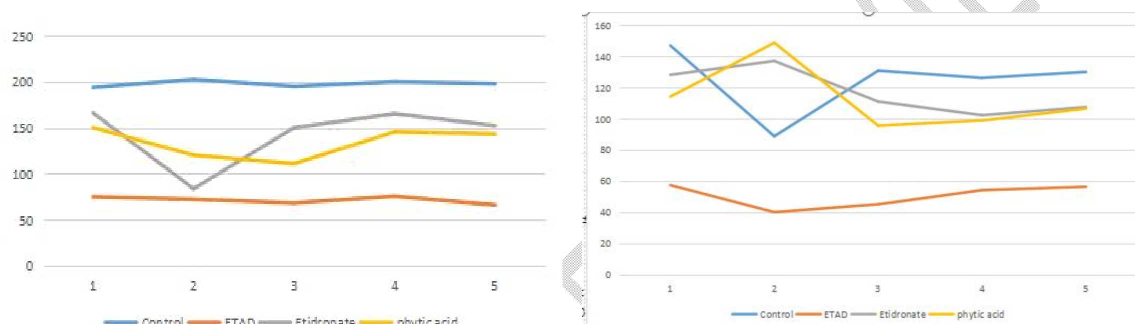
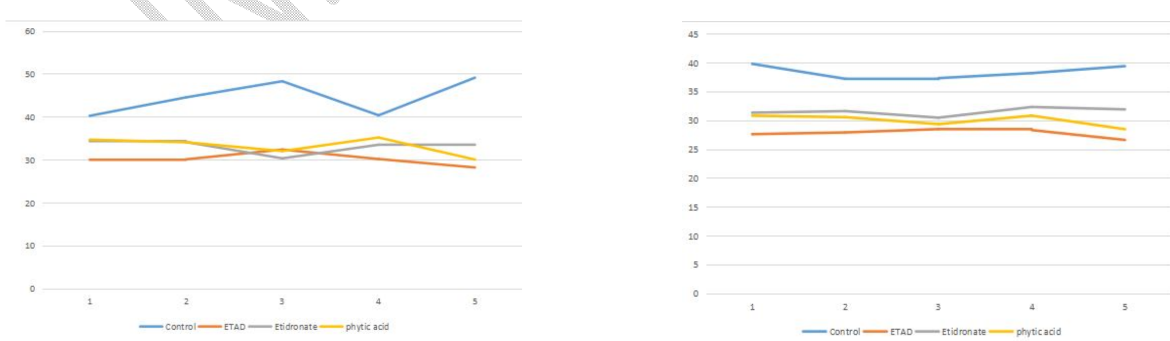


Fig. 4. GRAPHICAL REPRESENTATION OF MICROHARDNESS AT 10 MINUTES AND15 MINUTES



During root canal instrumentation, a smear layer may form, limiting the irrigating solution and inter-appointment drugs' entry into dentinal tubules.[12]

"Endodontic treatment requires removing the entire smear layer in the root dentin. Chelating agents can alter the chemical composition of dentin, affecting its microhardness, permeability, and solubility" [12]

Root canal therapy requires the use of chelating substances. The biocompatibility and capacity to remove smear layers should be considered while selecting an irrigation solution.[4]

"The effectiveness depends on the concentration and contact time.[3]Saline is not considered an effective irrigation agent due to its poor performance in terms of dissolving organic and inorganic material"[2]

To ensure consistent results, the flexural strength and surface hardness were determined using four radicular dentin sticks from the same root. Each of these sticks was allocated one of several experimental chelators, which were examined and compared to a saline (control).[1]

EDTA is the most commonly used chelator in root canal treatment. Using EDTA in combination with NaOCl in the absence of a distilled water dilution step may lower the amount of accessible chlorine in NaOCl.[2]

The current study found that Flexural Strength of EDTA is 71.26± 6.24 at 10 minutes which reduced to 50.61 ±0.72 at 15 minute.Whereas, the Flexure strength of Phytic acid is134.00±4.81 at 10 minutes which reduced to 112.83±5.43

The Flexure strength of Etidronic acid at 10 minutes was143.50±9.81 and at 15 minutes 117.25 14.26

Overall Flexural Strength of EDTA was considerably lower than untreated radicular dentin sticks, none of the tested irrigants, including Phytic acid and etidronate, significantly lowered the mechanical properties of dentin.But the reduction in Flexural Strength with the EDTA was more when compared with Etidronic Acid and Phytic acid.

Higher EDTA dilutions significantly reduced cell viability and morphology [4]. So, 18% Etidronate combined with saline is advised.[3] Phytic acid is beneficial in smear layer removal at a considerably lower concentration of 2.5% than EDTA, interpreting its biocompatibility.[3]

Phytic acid solution at 1% concentration is more successful than EDTA for chelation and smear layer removal, while maintaining biocompatibility [5]

For EDTA,surface microhardness at 10 minutes showed 30.16 ±1.27 which reduced to27.77 ±0.71 at 15 minutes.The surface microhardness of Phytic acid at 10 minutes was33.183±26 and 29.99±1.65 at 15 minutes.The surface microhardness of Etidronic acid at 10 minutes was 33.15±0.58 and 31.52±0.40 at 15 minutes.

In comparison to the control group, root dentin treated with HEBP showed the highest microhardness. This could be attributed to increased intertubular dentin surface available for hybridization when a gentle chelating irrigation regimen containing HEBP is applied.In comparison to EDTA,HEBP improved the binding of resin-based sealers to root canal dentin.[7]

Etidronic acid (HEBP) has minimal short-term effect on the action of NaOCl. Additionally, HEBP leads to slower demineralization compared to 17%. EDTA [13]

HEBP preserves the antibacterial properties of NaOCl solution. It contains antibacterial qualities of its own, which is an added bonus.[13]

Following the EDTA administration on dentin sticks, the dentin surface microhardness was considerably decreased

"EDTA's chelating activity effectively lowers dentinal microhardness. The degree of mineral content and the quantity of hydroxyapatite present in the intertubular substance are critical factors that influence the dentin structure's intrinsic hardness profile" [6]

Longer exposure to EDTA may promote dentine erosion and negatively impact the integrity of the dentine matrix. [5]

EDTA's removal of calcium ions (Ca^{2+}) from mineral tissues has been proven to degrade the dentin matrix. Two hours of exposure to 17% EDTA resulted in a depletion of calcium from the dentin surface down to approximately 150µm.[1]

EDTA has been linked to dentinal erosion due to its potential to demineralize root dentin when utilized over an extended period of time.[1]

According to Manu Unnikrishnan et al.(2019)[10], Irrigation regimen following the use of 2.5% NaOCl during instrumentation followed by application of 5-mL 17% EDTA solution for 1 min resulted in efficient smear layer removal and less decrease in dentin microhardness compared with 17% EGTA, 10% citric acid, and MTAD solution. This could be analternative way for using EDTA.

Longer the exposure to higher concentration,the Flexural Strengthand surface microhardness was reduced.

To prevent erosion, use chelators with low concentrations and shorter chelating durations, such as phytic acid, etidronate, and chitosan.[3]

According to the findings of De-Deus et al. (2006) [8]who claimed that the single use of 17% EDTA produced the greatest decrease in microhardness from reference state to 3 minutes, is in accordance with the present study.

Mukura Kulasekaran Dineshkumar et al. (2012)[7] also concluded in his study -Effect of ethylene diamine tetra-acetic acid, MTAD™, and HEBP as a final rinse on the microhardness of root dentin that EDTA showed less microhardness compared with other solutions.

F. Bosaid et al. (2020) [11] Concluded that, without significantly affecting the flexural strength of the root dentine, EDTA and 10% CA reduced the inorganic content and microhardness of the dentine surface, exposing more collagen and likely preventing additional collagen degradation by the remaining NaOCl.

According to Dentin Srinidhi Surya Raghavendra et al. (2018)[13] By employing etidronic acid and 0.2% chitosan solution, EDTA when used with NaOCl can be reduced. When combined with NaOCl, etidronic acid and 0.2% chitosan solution might be considered chelating agents that substitute EDTA.

According to Rama S kalluru et al.(2014) [14]Chelating agents EDTA, EDTAC drastically reduced the microhardness of root canal dentin, hence these agents should be used carefully.

CONCLUSION

Studies conducted throughout the years have mostly examined the effects of chemical agents and endodontic irrigating solutions used in adhesive dentistry on the mechanical characteristics and chemical composition of the coronal and root dentin.

Every chelator that was tested decreased the human radicular dentin's microhardness, but EDTA did so more dramatically than phytic acid.

It is clear from the study's limitations that the bulk and surface mechanical properties of radicular dentin are unaffected by chelators containing either 2.5% phytic acid or 18% etidronic acid.

Ethical Approval:

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

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REFFERENCES

1.Baruwa AO, Martins JN, Maravic T, Mazzitelli C, Mazzoni A, Ginjeira A. Effect of endodontic irrigating solutions on radicular dentine structure and matrix metalloproteinases—a comprehensive Review. Dentistry Journal. 2022 Nov 23;10(12):219.

2. Emre Erik C, OnurOrhanE,Maden M. **Qualitative analysis of smear layer treated withdifferent etidronate concentrations: A scanning electronmicroscopy study**. Microsc Res Tech. 2019;1–7. https://doi.org/10.1002/jemt.23318

3.El-Banna A, ElmesellawyMY,Elsayed MA**. Flexural strength and microhardness of humanradicular dentin sticks after conditioning with different endodontic chelating agents**. J Conserv Dent 2023;26: 344-8.

4.Nassar M, Hiraishi N, Tamura Y, Otsuki M, Aoki K, Tagami J. Phytic acid: An alternative root canal chelating agent. Journal of endodontics. 2015 Feb 1;41(2):242-7.

5.Deniz Sungur D, Aksel H, Ozturk S, Yılmaz Z, Ulubayram KE. Effect of dentine conditioning with phytic acid or etidronic acid on growth factor release, dental pulp stem cell migration and viability. International Endodontic Journal. 2019 Jun;52(6):838-46.

6. Nikhil V, Jaiswal S, Bansal P, Arora R, RajS, Malhotra P. **Effect of phytic acid, ethylenediaminetetraacetic acid,and chitosan solutions on microhardness of the human radicular dentin.**JConserv Dent 2016;19:179-83.

7. Dineshkumar MK, VinothkumarTS,Arathi G, Shanthisree P, Kandaswamy D. **Effect of ethylenediamine tetra-acetic acid, MTADTM , and HEBP as a final rinse onthe microhardness of root dentin**. J Conserv Dent 2012;15:170-3

8. Irina Tsenova-Ilieva, Emilia Karova Effect of Endodontic Irrigants on Root Dentin Microhardness: A Systematic Review. International Journal of Science and Research (IJSR)2020.9(4).491-496

9.Irina Tsenova-Ilieva, Emilia Karova.Antonio M. Cruz-Filho. Effect of Chelating Solutions on the Microhardness of Root Canal Lumen Dentin. JOE. 2011 Mar;37(3).

10.Unnikrishnan M, Mathai V, Sadasiva K, Santakumari RS, Girish S, Shailajakumari AK. The evaluation of dentin microhardness after use of 17% EDTA, 17% EGTA, 10% citric acid, MTAD used as chelating agents combined with 2.5% sodium hypochlorite after rotary instrumentation: An in vitro SEM study. J Pharm Bioall Sci 2019;11:S156-63.

11.Bosaid F, Aksel H, Makowka S, Azim AA. Surface and structural changes in root dentine by various chelating solutions used in regenerative endodontics. International Endodontic Journal. 2020 Jul 30;53(10):1438–45.

12.Rajakumaran A, Ramesh H, Ashok R, et al. (July 25, 2019) Smear Layer Removal and Microhardness Alteration Potential of a Naturally Occurring Antioxidant – An In Vitro Study. Cureus 11(7): e5241. DOI 10.7759/cureus.5241

13.Srinidhi Surya Raghavendra. Effect of Etidronic Acid, Chitosan and EDTA on Microhardness of Root Canal Dentin. Saudi Journal of Oral and Dental Research (SJODR). 2018 Apr 15;

14.Kalluru RS. Comparative Evaluation of the Effect of EDTA, EDTAC, NaOCl and MTAD on Microhardness of Human Dentin – An In-vitro Study. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2014;