

Effect of the Chelating Agent Alendronic Acid versus EDTA on the Physicochemical Properties of Dentine

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ABSTRACT

Objective: The present study aimed to evaluate the changes in the physicochemical properties of dentine after irrigation with a solution of 0.22% alendronic acid (AA) as a chelating agent compared to 17% ethylenediaminetetraacetic acid (EDTA).

Methods: A total of 48 extracted premolars and molars that were intact, free of caries or cracks, without root canal treatment and restorations were collected. The roots were randomised into three groups (n=16): Group A: Distilled Water (dH₂O); Group B: 17% EDTA, and Group C: 0.22% AA. Longitudinal sections of the dentine with a root of 1x1x10 mm were made with a diamond disc and a low-speed handpiece for bending tests (n=9). For morphological analysis, images were taken with a scanning electron microscope, crystallographic analysis with X-ray diffraction, and chemical analysis with Fourier Transform Infrared Spectroscopy (FTIR) and Vickers Hardness. For this purpose, cross-sections were made through the root using the Isomet to obtain 3 mm thick dentine discs (n=14). The samples were stored in dH₂O for up to 24 h before use and dried at room temperature before exposure to chelating solutions for 1 h in a Stuart STR6D mixer at 50 rpm. For data comparison, the Kruskal-Wallis statistical test was used ($\alpha=0.05$).

Results: The chelating solutions of EDTA and AA cause alterations in the physicochemical structure of dentine, attacking mainly the inorganic part (Hydroxyapatite), which was observed in the decrease in intensity of the peaks in the X-ray diffraction pattern of hydroxyapatite. This generated a greater exposure of the collagen fibres that were observed in SEM and the increase in the bands characteristic to Collagen Type I in the infrared spectrum at 1645, 1550, and 1240 cm⁻¹ belonging to amide I (C=O), amide II (N-H) and amide III (C-N), significantly affecting its dentine hardness ($p=0.001$).

Conclusion: AA can be used as a chelating agent in the area of dentistry. It does not generate a significant demineralising effect that modifies the physicochemical properties of dentine, as observed with EDTA.

Keywords: Alendronic acid, chelating solution, EDTA, flexural strength, vickers hardness

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HIGHLIGHTS

- Alendronic acid solution 0.2% is a potent chelating agent that does not modify the hardness of dentine.
- The solution generates minimal changes in chemical properties and does not cause the decalcification of dentine.
- An alendronic acid solution of 0.2% produces minimal erosion on the dentine surface compared to 17% EDTA.
- Alendronic acid solution 0.2% has the potential to be proposed as an alternative to 17% EDTA.

INTRODUCTION

The success of root canal treatment is associated with instrumentation and chemical-mechanical irrigation for disinfection and shaping of the root canal before three-dimensional filling. During root canal instrumentation, the formation of a residual layer known as the smear layer is inevitable. This layer consists of organic and inorganic debris, including dentinal particles, remnants of pulp tissue, and potential microbial contaminants (1). Therefore, irrigation with biocompatible, cost-effective solutions during instrumentation is essential for the elimination of necrotic tissue, reduction of bacterial load, decontamination of areas where files cannot be accessed, elimination of the smear layer, and lubrication of the root canal (2, 3). However, there is currently no irrigating solution that meets all these characteristics. Therefore, it is necessary to irrigate with a sequence of irrigating agents, including agents with chelating properties, to remove the smear layer, because it prevents the adhesion capacity of the cement sealer.

Ethylenediaminetetraacetic acid (EDTA) and citric acid are the most commonly used strong chelating solutions in the endodontic area (3–5). However, their action is not limited to the smear layer and causes demineralisation, leading to changes in the physicochemical properties of dentine, which is a hard-mineralised tissue composed of Type I collagen with hydroxyapatite and carbonates that provide rigidity and resistance. These changes in the properties of dentine can have a negative effect on restoration, leading to fracture and compromising the adhesion of the restorative material (6, 7). To achieve this aim, alternative chelating solutions have been evaluated, such as chemical compounds from the bisphosphonate family approved by the Food Drug Administration (FDA) such as etidronate (1-hydroxyethylidene-1,1-disphosphonate) and alendronic acid ((4-amino-1-hydroxybutane-1,1-diyl) bis (phosphonic acid)-AA) which contain phosphate groups that give them the ability to act as a chelating agent and bind to transition metal ions through coordination bonds (7, 8). In the case of etidronate, it was proposed by Zehnder et al. (9), within a continuous irrigation protocol in combination with sodium hypochlorite (NaOCl), giving rise to various investigations. In the case of AA, it has only been reported as a promising new chelating agent due to its effectiveness in eliminating intracanal medication (10). However, the lack of literature highlights the need for studies focused specifically on evaluating its effects on the changes that it could generate in the physicochemical properties of dentine after exposure. Therefore, the purpose of this study was to evaluate the effect of EDTA and AA chelating solutions through a comparison of their impact on flexural strength, Vickers hardness, and changes in chemical composition and crystallinity. The null hypothesis of this study was that no differences were observed in the physicochemical properties of dentine in samples irrigated with EDTA and AA.

MATERIALS AND METHODS

This research was conducted in full accordance with the World Medical Association Declaration of Helsinki and has been approved by the Research Ethics Committee of the Faculty of Stomatology, UASLP, with code: CEI-FE-033-023 (approved on 04/05/2023).

Preparation of 0.22% AA Solution

The 0.22% AA solution was prepared by dissolving 4 Dronadil® tablets (Laboratorio Alparma, Ciudad de México, México) with 0.070 g of the active ingredient (AA) in 130 mL of dH₂O and adjusting the pH to 7 (0.195 g/tablet).

Preparation of 17% EDTA Solution

The 17% EDTA solution was prepared by dissolving 17g EDTA (Hycel; Jal, México) in 100 mL of dH₂O and adjusting the pH to 7 (9.25 mL 5 M sodium hydroxide) (11).

Sample Preparation

48 recently extracted molars and premolars that were intact, free of caries or cracks, and without root canal preparation and restorations were collected and stored in dH₂O until further use. Teeth with curved roots, anatomical or morphological deformities, resorptions, cervical abrasions, calcifications, cracks or fractures and immature apices were excluded. The teeth were randomly divided into three groups (Group A: dH₂O; Group B: 17% EDTA and Group C: 0.22% AA). For X-ray diffraction, FTIR, and Vickers hardness tests, 3mm thick vertical coronal dentine discs (one buccal and one lingual/palatal) were obtained from each tooth using the Isomet (Buehler, Illinois, USA). For mechanical analysis, 1×1×10 mm dentine blocks were made longitudinally with a diamond disc and a low-speed handpiece. Each dentine block and disc were measured with a Digital Vernier (Mitutoyo UK Ltd, Andover, Hampshire, UK) with a precision of 0.01 mm. The samples were washed with dH₂O in a sonicator bath for 4 min (5 washes, until a translucent liquid was obtained, each with a volume of 40 mL) to eliminate the smear layer generated by the cuts. They were stored in dH₂O, and 24 h before use, they were dried at room temperature before being exposed to chelating solutions for 1 h in a mixer (Stuart, STR6D, Delaware, USA) at 50 rpm Figure 1 shows the flowchart of the experimental procedures of the study). For data comparison, the Kruskal-Wallis statistical test was used ($\alpha=0.05$).

Morphological Characterization

For Scanning Electron Microscope (SEM), three roots were randomly selected and divided into two halves along the coronal-apical axis. Grooves were made along the longitudinal axis of each root using a diamond blade and a low-speed handpiece. The roots were then divided longitudinally into two halves using a chisel and mallet. Dentine morphology was evaluated using a scanning electron microscope (SEM; JSM-6510, JEOL, Tokyo, Japan) operating at 10kV, and images of the top surfaces of each root were captured at 3500x and 7000x magnification (two areas from each root were randomly selected and analysed in the coronal third). For this, the samples were dehydrated in serial amounts of 20 to 100% alcohol for 10 min. The samples were cut in half and placed vertically on pins using carbon tape with double adhesive and coated in gold for 40 s.

Chemical Analysis

The chemical changes generated by the irrigating agents were evaluated using the Fourier Transform Infrared Spectroscopy (FTIR) in the attenuated total reflectance mode (ATR) in the intermediate infrared region from 4000 to 400 cm⁻¹ (Thermo

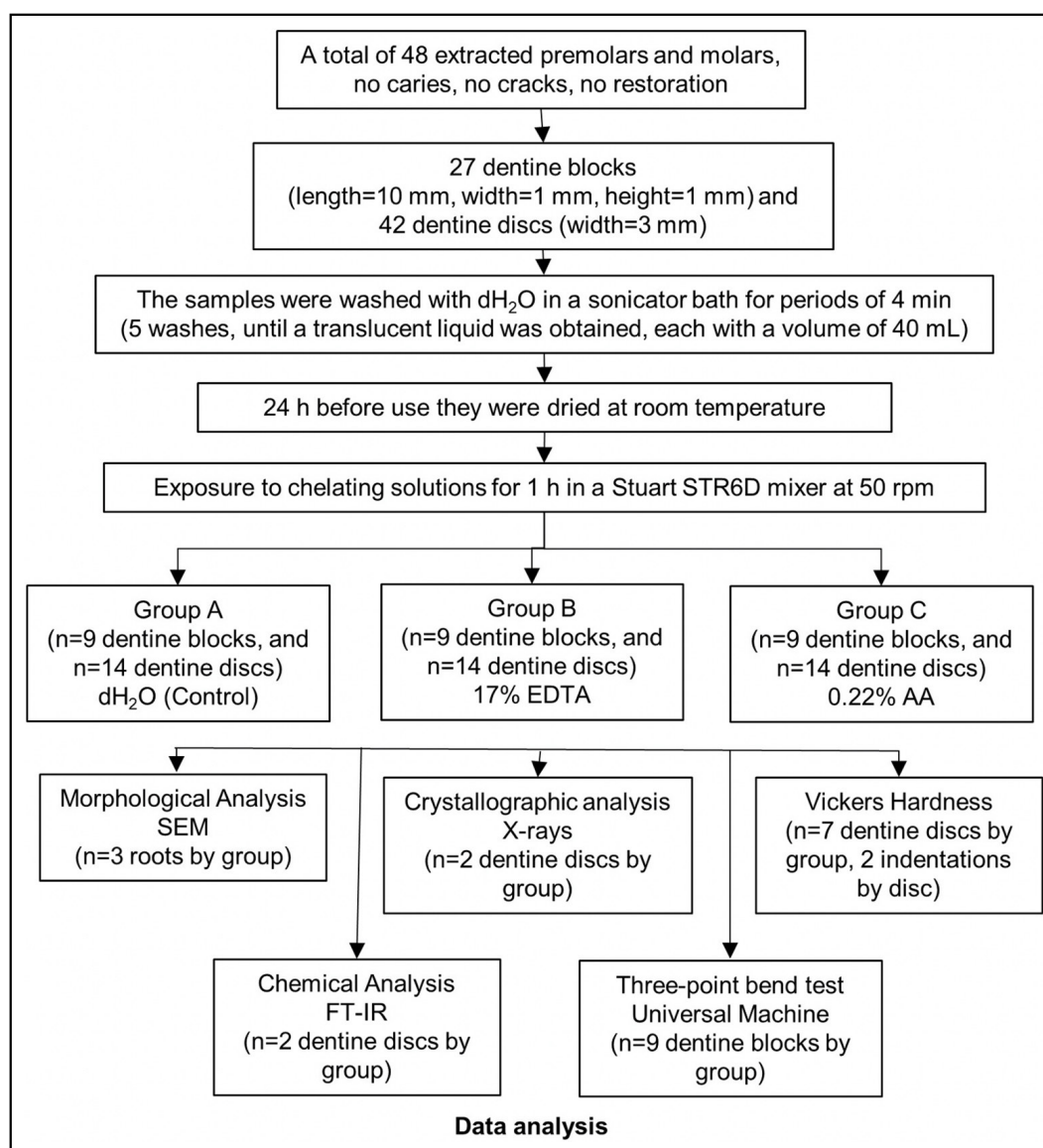


Figure 1. The flowchart of the experimental procedures of the study.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

Fisher Scientific, Nicolet IS50, Waltham, USA), at a resolution of 4 cm⁻¹ and 32 scans. For FTIR-ATR, two discs were randomly selected from each experimental group.

X-ray Diffraction Test

Changes in the crystalline structure on the dentine surface after irrigation were evaluated by X-ray diffraction. Post-irrigation dentine discs were analysed on a PANalytical X'Pert PRO X-ray diffractometer (PANalytical; Empyrean, Houston, TX, USA) with a Cu-Kα monochromatic radiation source at a wavelength of 1.54 Å in a range of 10 to 70° in 2θ, at intervals of 0.02° and an acquisition time of 12 s/step. They were also analysed using the X'Pert HighScore Plus PANalytical software. For the X-ray diffraction test, two discs were randomly selected from each experimental group.

Mechanical Analysis

The sample size per group for the mechanical tests was calculated using G*Power v.3.1.9.7 software for Windows 10

(Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany) using the Wilcoxon-Mann-Whitney test. An alpha error of 0.05, the beta power of 0.95, and an N2/N1 ratio of 1 were considered. The test calculated a total of 9 samples/group. The mechanical changes generated by the action of the irrigants were evaluated in a universal testing machine (Shimadzu, AGS-X500, Carlsbad, USA) using a three-point bending test. 1x1x10 mm dentine bars were evaluated at a speed of 1mm/min and with a load of 0.1N.

Vickers Hardness

Vickers test was performed to measure the surface hardness of dentine after irrigation. Indentations were made 0.5 mm from the root canal space using the Shimadzu Vickers durometer (Digital Microhardness Tester, HMV-G, Mammelzen, Germany) with a load of HV 0.1 (980.7 mN) for 10 s. To increase the power of the Vickers hardness test, the decision was made to increase the sample size to 14 measurements per group (n=7 dentine discs by group, 2 indentations per disc).

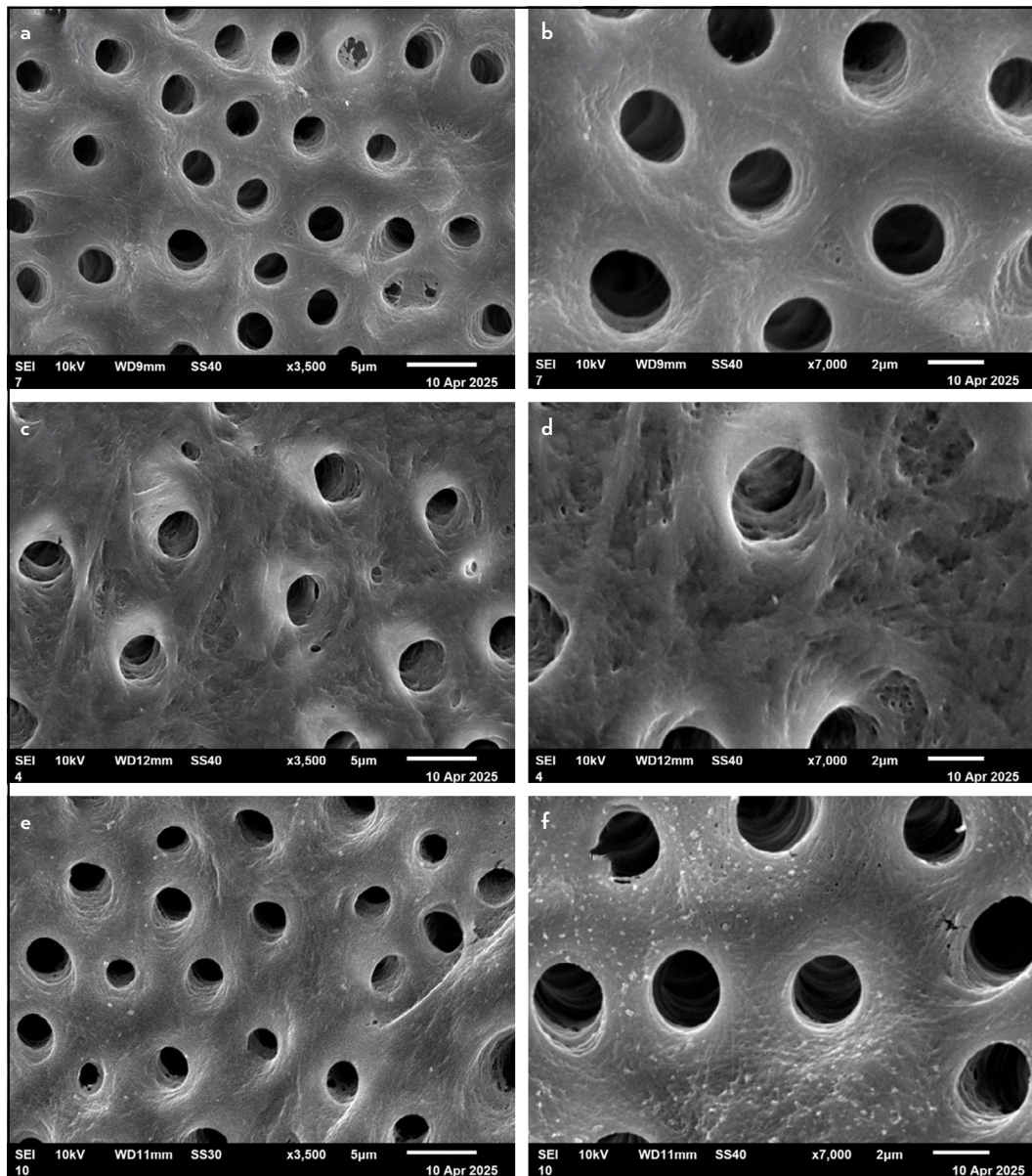


Figure 2. Scanning electron microscopy (SEM) images of the surface with secondary electrons. (a, b) dH₂O, (c, d) EDTA and (e, f) AA at 3500x and 7000x magnification.

EDTA: Ethylenediaminetetraacetic acid.

Statistical Analysis

The statistical analysis was performed using the Minitab software version 18 (Minitab, LLC is a privately owned company headquartered in State College, State College, PA, USA), where the Shapiro-Wilk normality test was performed to evaluate the normality of the data, where the non-normality of the data was determined. After this, the Kruskal-Wallis statistical test was performed ($\alpha=0.05$). The flow chart of the experimental procedures is presented in Figure 1.

RESULTS

Morphological Characterization

Micrographs obtained by SEM, show the morphological changes in the surface of the dentine after irrigation (Fig. 2). In Group B (Fig. 2c, d), the exposed collagen fibres are observed in the intertubular dentine. While in Group A (Fig. 2a, b), the

morphology is observed with a smooth surface, free of exposed collagen fibres, like that of Group C (Fig. 2e, f).

Chemical Analysis

After baseline correction and normalization, the FTIR spectra were analysed. Figure 3 shows the infrared spectrum of dentine after irrigation with irrigating solutions. In Group A (Fig. 3a), bands corresponding to the asymmetric stretching of the phosphate group (PO_4^{3-}) are observed around 1033 cm^{-1} , which constitutes hydroxyapatite. Likewise, another characteristic band of the phosphate group at 962 cm^{-1} is associated with symmetric stretching. A medium-intensity band is observed in the region $565\text{--}566\text{ cm}^{-1}$ and at $603\text{--}604\text{ cm}^{-1}$, associated with an asymmetric bending of the O-P-O bond (12). Peaks in the region of $960\text{ to }1100\text{ cm}^{-1}$, and the bands of $603\text{ and }565\text{ cm}^{-1}$ correspond to the decrease in the phosphate of hydroxyapatite in Group B (Fig.

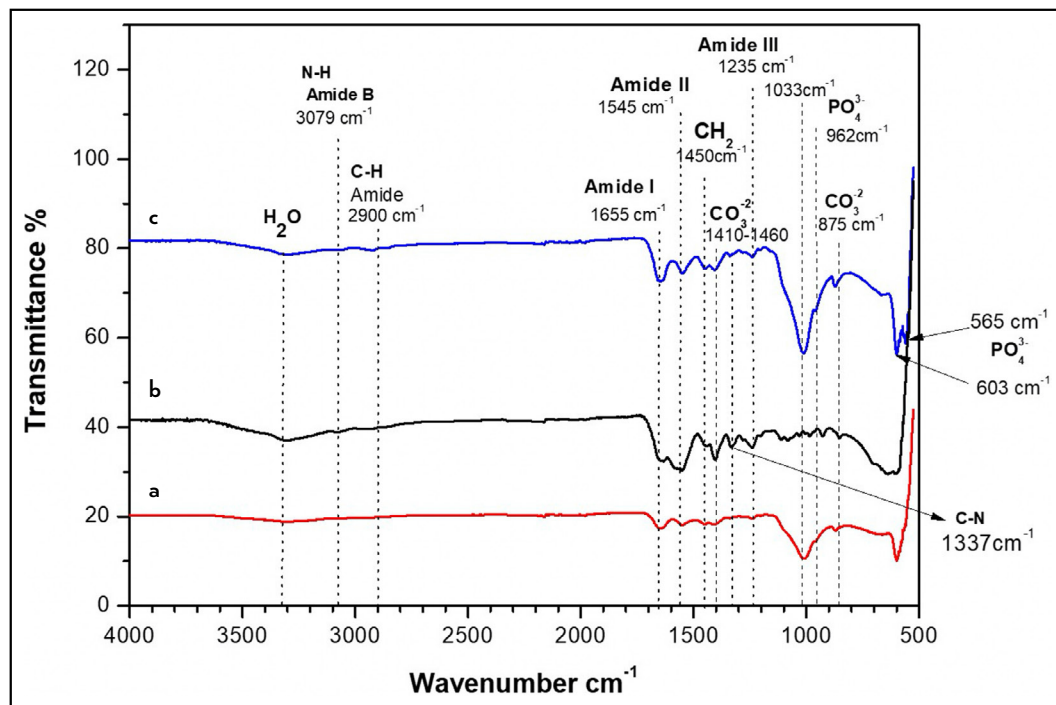


Figure 3. Fourier transform infrared spectrum of dentine after irrigation with chelating solutions. (a) dH₂O, (b) EDTA and (c) AA.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

3b). Regarding Collagen Type I, another constituent of dentine, characteristic collagen bands can be observed around 1655, 1550, and 1235 cm⁻¹, which belong to amide I (C=O), amide II (N-H) and amide III (C-N) (12). Also, a shoulder is observed at 3079 cm⁻¹ associated with free N-H stretching for amide II. Around 2900 cm⁻¹, another band appears associated with asymmetric C-H stretching of the amide group (13, 14). The CH₂ group appears at 1450 cm⁻¹ with a deformation vibration (12, 13). Regarding the characteristic bands of amide I, the vibration of the amide carbonyl groups along the polypeptide backbone can be found (15) between 1650–1680 cm⁻¹, which correspond to the vibration of C=O bonds, a C-N stretching and a N-H deformation usually reported at 1653 cm⁻¹ (13).

Amide II, with a stretching movement of the C-N bond and a N-H swing, presents a characteristic band at 1545 cm⁻¹ to the amide groups of the collagen triple helix. Amide III is found between 1240–1242 cm⁻¹ and corresponds to the N-H deformation associated with tertiary amines. Therefore, the ratio between Amide III/Amide I can be used to infer the relationship between carbon and nitrogen present in collagen. In addition, a medium intensity band associated with C-N stretching is also observed at 1337 cm⁻¹. Regarding the infrared spectrum, the ratio of Amide III (1235 cm⁻¹), CH₂ (1450 cm⁻¹) and CO₃²⁻ group (1410 cm⁻¹) increased in intensity in Group B, due to the loss of inorganic structure, as well as a stretching and amplitude of the Amide II peak (1545 cm⁻¹), which corresponds to the changes in N-H and the stretching of the average intensity of C-N. To the spectrum of Group C, only an increase in the Amide I signal is observed.

X-ray Diffraction Test

The crystallographic properties of dentine were evaluated by post-irrigation X-ray diffraction. The diffraction pattern of each of the treatments can be seen in Figure 4. It can be observed that Group A presents peaks of greater intensity at 25.89, 32.05, 39.67, 46.78, 49.49, and 53.54 in 2θ. According to the X'Pert HighScore Plus PANalytical software database, the pattern corresponds to the crystallographic card 00-001-1008 of the JCPDS-International Centre for Diffraction Data database, and according to the Crystallography Open Database with card 1521038, both correspond to a hydroxyapatite in which the main peaks correspond to the crystallographic planes (002), (210), (211), (112), (022), (310), (222) and (213) respectively (16). Type I collagen, one of the other main components of dentine, corresponds to a low crystallinity polymer. Regarding Groups A and B, a decrease in the intensity of the peaks of the crystalline planes can be observed, indicating a reduction in the degree of crystallinity, possibly due to the loss of Ca²⁺ ions.

Mechanical Analysis and Vickers Hardness

Table 1 shows the results obtained from the bending test, in which it is observed that the irrigating solutions did not produce significant effects on the bending of the dentine (35.32, 35.41 and 33.39N, respectively). According to the Kruskal-Wallis statistical test using the Minitab software version 18 (Minitab, LLC is a privately owned company headquartered in State College, State College, PA, USA), no statistically significant differences were found (p=0.409). However, regarding the hardness of the dentine, a decrease of 43.7% was observed when it was irrigated with EDTA (25.53 HVN), compared to the control sample (54.92 HVN). According to the Kruskal-Wallis statistical test, statistically significant differences were found (p=0.001).

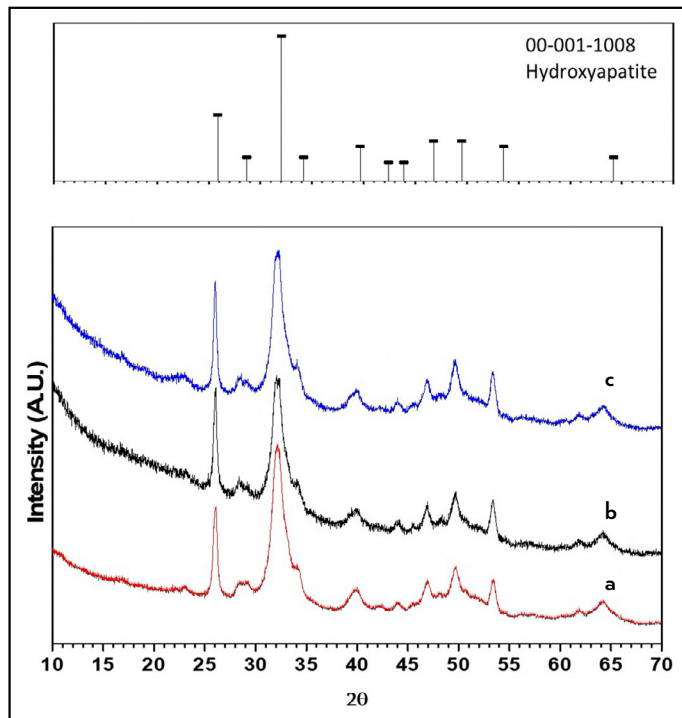


Figure 4. Diffraction pattern of dentine after irrigation with chelating solutions. (a) dH₂O, (b) EDTA and (c) AA.

EDTA: Ethylenediaminetetraacetic acid, AA: Alendronic acid.

DISCUSSION

Dentine is the principal dental structure that absorbs mechanical loads (17). Its chemical composition is up hydroxyapatite crystals, which represent 70% of dentine and correspond to the mineral part, as well as 20% organic matrix in the form of type I collagen and 10% water (18, 19). Dentine is presented in the form of a dense collagen network covered by hydroxyapatite (20). Collagen is a fibrous and insoluble protein composed of three polypeptide chains formed by the repetition of amino acids such as glycine, proline, and hydroxyproline, and the union of the 3 chains occurs through hydrogen bonds forming the triple helix (21). Dentine hydroxyapatite is composed of smaller calcium phosphate crystals, richer in carbonates and poorer in calcium, for which the chemical formula is Ca₁₀(PO₄)₆(OH)₂ (22).

Histologically, dentine contains dentinal tubules, which are cylindrical structures that extend from the pulp to the amelo-

dentinal or cementodentinal junction, and house the odontoblastic processes. These tubules are covered by peritubular dentine, which in turn are joined together by intertubular dentine composed of a matrix of collagen and apatite fibres. For Intertubular dentine, much of the dentine and its structure is made of collagen fibres at right angles to the tubule, in which hydroxyapatite crystals are deposited (23). These channels vary in number and can increase as they are closer to the pulp, which gives it anisotropic behaviour, that is, its properties are different depending on the direction. Therefore, this composition and structure of dentine provide it with three physical properties, namely hardness, resistance, and elasticity, which are essential to understanding masticatory function.

In endodontic treatment, the elimination of microorganisms from the root canal system is essential. In addition to mechanical instrumentation, chemical disinfection using irrigating solutions plays a crucial role. (2, 3). In the instrumentation processes, a layer is created that covers the walls of the canal, composed mainly of remains of dentine and pulp tissue, and microorganisms, called the smear layer (24). Therefore, using irrigating solutions is essential, and among the most used in endodontic treatment is sodium hypochlorite (NaClO), due to its bactericidal effect and ability to dissolve organic matter and necrotic tissue (24). Therefore, when used as an irrigating solution in the preparation of the canal, the dentine walls are left with fewer organic remains but are covered with inorganic particles (25). To remove the inorganic components of the dentine barrel, it is important to combine it with a chelating solution such as EDTA. This was reported by the study of Ligeng Wu et al. (26), where combinations of NaOCl with chelating agents were performed, and it effectively removes the dentine smear layer. However, the physicochemical properties of dentine can be affected. This causes decalcification of the dentine of the canal, which can increase when using strong chelating solutions, which can compromise the sealing of the root canal (25, 27).

The hardness of dentine was evaluated by the Vickers hardness test, which is a universal test that uses a diamond indenter to affect the sample at a defined force, allowing the hardness of the material to be measured, since the larger the indentation, the softer the material. Regarding the results of this research, the hardness decreased in Group B, which was irrigated with EDTA, from 58.45 to 25.53 HVN, which represents a statistically

TABLE 1. Median, first quartile (Q1) and third quartile (Q3) of the flexural strength and microhardness (VHN) of dentine after irrigation with chelating agents

Specimen	n	Flexural strength		n	HVN	
		Median	Q1-Q3		Median	Q1-Q3
Group A	9	35.32	31.84–52.08	14	54.925	52.83–61.53
Group B	9	35.41	32.61–38.83	14	25.535*	23.15–28.83
Group C	9	33.39	28.04–37.60	14	58.450	39.68–63.93
p		0.409			0.001	

The Kruskal-Wallis test; *: Indicates a significant difference between the control group and the chelating agent (vertical) group ($p < 0.05$). VHN: Vicker's hardness number, HVN: Vickers hardness number

significant change in said property of dentine. However, these significant changes were not appreciated in Group C. Furthermore, the action of EDTA is not self-limiting, meaning that its chelating effect continues until all the available solution forms complexes between the salt and the calcium of the dentine, generating a chemical change in the dentine. This was corroborated by FTIR, by decreasing the bands associated with hydroxyapatite, as well as the bands that appear at 1100 cm^{-1} and 1005 cm^{-1} of low intensity, which are identified by the deformations of the C-O-H, C-O, and C-O-C groups of carbohydrate residues and out-of-plane torsions of carboxylic acids (15). According to the studies reported by Barón in 2020, they are associated with the amide III/ CH_2 that increased significantly, and this is because dentine is richer in collagen after exposure to EDTA (13). These facts are corroborated in the micrographs obtained with the SEM, where there is a decrease in hydroxyapatite (inorganic matter), which covers the collagen fibres, which are mostly exposed when the hydroxyapatite is lost due to the effect of these solutions.

This is in accordance with the study carried out by Liñan et al. (28), where they observed the degree of erosion of EDTA in the different thirds. The presence of dentinal canaliculi can be seen due to the destruction of peritubular and intertubular dentine.

Likewise, in 2002, Calt and Serper reported that EDTA causes demineralisation effects on peritubular and intertubular dentine over time (29). Other studies show that the use of EDTA results in the elimination of the dentine barrel, but results in a demineralisation of the superficial dentine of 4 to $6\text{ }\mu\text{m}$ in the coronal, middle, and apical third of the canal (30). Furthermore, these changes were confirmed in the tests of the analysis of the crystallinity of the dentine, since they are related to the changes observed in the diffraction patterns. In X-ray diffraction, a decrease in the crystallinity of the dentine is observed where there is a decrease in the signal of the crystalline planes of hydroxyapatite, which is the part that mainly provides hardness to dentine, since it is a biocrystal composed of calcium, phosphorus and hydrogen atoms.

However, although dentine is less mineralised than enamel, collagen is what gives it the capacity for compression and traction, so in the bending test, statistical changes were not appreciated (31). These physicochemical changes of dentine after exposure to chelating solutions are due to minerals present in dentine, mainly phosphate and calcium, which are soluble in water. The chelating interacts with the calcium ions present in the structure of hydroxyapatite, generating complexes and not interacting with the organic part of dentine (13). These changes generated by EDTA have been reported by Beltz et al. (32), where they observed that NaOCl dissolves 90% of the organic components of dentine, and EDTA at 17% dissolves 70% or more of the inorganic matrix. EDTA is a strong chelating agent because it has six potential sites in its chemical structure to bind to metal ions, in addition to its high concentration. In contrast, AA is a compound of the bisphosphonate family, for which the mechanism of action is an antiresorptive effect, that is, it prevents the function of osteo-

clasts, decreasing bone resorption. It is characterized by having two phosphoric groups and an amine group in its chemical structure, in addition to its hydroxyl group (OH)-, which gives it the property of a strong chelating agent, attracting metal ions. However, the AA solution used in this work, as well as being an FDA-approved compound, does not affect the physical properties of dentine. Since its toxicity is low, its clearance from plasma and soft tissues is fast, making it easy for the kidney to eliminate it quickly. In addition, its absorption decreases when taken with food, especially if it contains calcium, iron, coffee, or tea. Between 20–80% is deposited in the bone, the rest is excreted in the urine (31).

Bisphosphonates are synthetic compounds with structural characteristics similar to pyrophosphates, so they bind to the hydroxyapatite of the bone, where the oxygen element (P-O-P) is replaced by the carbon in the bisphosphonate (P-C-P), which makes it resistant to hydrolysis. Therefore, the solubility of hydroxyapatite is reduced by this double phosphoric acid, which reduces osteoclast activity, decreases bone resorption, and stimulates osteoblasts to produce inhibitors of osteoclast formation.

Similarly, some studies have been carried out using chemical compounds of the bisphosphonate family as chelating agents in dentistry, such as those carried out by Lottanti et al. (25), where they evaluated the effect of EDTA, etidronic acid, and peracetic acid. It showed that EDTA presents greater demineralisation at 3 min, while Etidronic acid used as a final irrigant with NaOCl did not show demineralisation of the canal dentine. De-Deus et al. (33) compared the chelating effect of Etidronate at 9 and 18% and EDTA at 17%, reporting that after 60 s all the smear is eliminated. However, an increase is shown in the size of dentinal tubules over time, characteristic of demineralisation, with a faster activity than bisphosphonate.

This study has some limitations. One of them is the anisotropic characteristics of dentine, which can influence mechanical properties such as hardness and tensile strength. The contact time and volume of the irrigation solution used during root canal treatment may be less than those used in the trial. However, each group was subjected to the same conditions, as well as the randomisation of the teeth, allowing for comparable results among the chelating solutions evaluated. Another important aspect is the need for further *in vitro* and *in vivo* studies to evaluate the efficacy of the AA chelating agent in removing the smear layer from root canal walls and its antibacterial efficacy. This also includes an assessment of the implications this irrigating agent may have on restoration processes (34, 35).

CONCLUSION

EDTA and AA chelating solutions cause alterations in the physicochemical structure of dentine, attacking mainly the inorganic part (Hydroxyapatite). These alterations cause greater exposure of collagen fibres, with the EDTA solution being where the greatest changes are generated. However, these alterations do not compromise the bending of dentine, but rather its hardness when irrigated with EDTA. It is also

concluded that AA can be used as a chelating agent in the dentistry area since it does not have a significant demineralising effect that modifies the physicochemical properties of dentine, as observed with EDTA.

Disclosures

Ethics Committee Approval: The study was approved by the Research Ethics Committee of the Faculty of Stomatology, UASLP (no: CEI-FE-033-023, date: 04/05/2023).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Use of AI for Writing Assistance: The development of this work does not involve the use of technologies assisted by artificial intelligence (AI) (such as Large Language Models [LLMs], chatbots, or image creators) and assert that there is no plagiarism in their paper, including in text and images produced by the AI.

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REFERENCES

- Karthikeyan HR, Rajakumaran A, Rajendran MR, Balaji L. Evaluation of Effect of Natural Extract Sodium Gluconate on Smear Layer and Dentine Decalcification Compared with EDTA - An *In-vitro* Study. *Eur Endod J* 2023; 8(4):274–79. [\[Crossref\]](#)
- Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. *Dent Clin* 2010; 54(2):291–312. [\[Crossref\]](#)
- Prabjot K. Role of Irrigants in Endodontics. *J Dent Probl Solut* 2020; 7(2):100–04. [\[Crossref\]](#)
- Hulsmann M, Heckendorff M, Lennon Á. Chelating agents in root canal treatment: Mode of action and indications for their use. *Int Endod J* 2003; 36(12): 810–30. [\[Crossref\]](#)
- La Rosa GRM, Plotino G, Nagendrababu V, Pedullà E. Effectiveness of continuous chelation irrigation protocol in endodontics: a scoping review of laboratory studies. *Odontology* 2024; 112(1):1–18. [\[Crossref\]](#)
- Ratih DN, Widyastuti A, Monika A. Effect of Final Irrigation Solutions on Mechanical Properties of Root Canal Dentine Running title: Final Irrigation on Mechanical Properties. *Eur Endod J* 2024; 9(4):405–10. [\[Crossref\]](#)
- Marques JA, Falacho RI, Fateixa S, Caramelo F, Santos JM, Rocha J, et al. Advancing Adhesive Strategies for Endodontically Treated Teeth-Part I: Impact of Endodontic Irrigation Protocols on the Chemical Composition and Structural Integrity of Coronal Dentin. *J. Esthet. Restor. Dent* 2025; 37(7):1848–64. [\[Crossref\]](#)
- Mollaamin F, Monajjemi M. Application of DFT/TD-DFT Frameworks in the Drug Delivery Mechanism: Investigation of Chelated Bisphosphonate with Transition Metal Cations in Bone Treatment. *Chem* 2023; 5(1):365–80. [\[Crossref\]](#)
- Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in Root Canal Therapy Reconsidered. *J Endod* 2005; 31(11):817–20. [\[Crossref\]](#)
- Mejía-Haro R, Méndez-González MV, Zavala-Alonso NV, Ortiz-Magdaleno M, Gutierrez-Sánchez M. Efficacy of alendronic acid solution in removal calcium hydroxide from root canals. *J Clin Exp Dent* 2024; 16(5): e595–601. [\[Crossref\]](#)
- Hahn FL, Reygadas F. Demineralization of hard tissues. *Science* 1951; 114(2966): 462–63. [\[Crossref\]](#)
- de Miranda RR, Silva ACA, Dantas NO, Soares CJ, Novais VR. Chemical analysis of *in vivo*-irradiated dentine of head and neck cancer patients by ATR-FTIR and Raman spectroscopy. *Clin Oral Investig* 2019; 23: 3351–58. [\[Crossref\]](#)
- Barón M, Morales V, Fuentes MV, Linares M, Escribano N, Ceballos L. The influence of irrigation solutions in the inorganic and organic radicular dentine composition. *Aust Endod J* 2020; 46(2): 217–25. [\[Crossref\]](#)
- Saska S, Teixeira LN, Tambasco De Oliveira P, Minarelli Gaspar AM, Lima Ribeiro SJ, Messaddeq Y, et al. Bacterial cellulose-collagen nanocomposite for bone tissue engineering. *J Mater Chem* 2012; 22(41): 22102–12. [\[Crossref\]](#)
- Riaz T, Zeeshan R, Zarif F, Ilyas K, Muhammad N, Safi SZ, et al. FTIR analysis of natural and synthetic collagen. *Appl Spectrosc Rev* 2018; 53(9):703–46. [\[Crossref\]](#)
- Arellano Ramírez ID, Ramírez VR, Acevedo NA, Parra ER, Medina CDA. Influence of the calcination temperature on the crystallographic, compositional and morphological properties of natural hydroxyapatite obtained from sheep bones. *Sci Tech* 2021; 26(4): 525–31. [\[Crossref\]](#)
- Lainović T, Margueritat J, Martinet Q, Dagany X, Blažić L, Pantelić D, et al. Micromechanical imaging of dentin with Brillouin microscopy. *Acta Biomater*. 2020; 105:214–22. [\[Crossref\]](#)
- Wu XT, Mei ML, Li QL, Cao CY, Chen JL, Xia R, et al. A direct electric field-aided biomimetic mineralization system for inducing the remineralization of dentin collagen matrix. *Mater* 2015; 8(11):7889–99. [\[Crossref\]](#)
- Ghilotti J, Fernández I, Sanz JL, Melo M, Llena C. Remineralization Potential of Three Restorative Glass Ionomer Cements: An *In Vitro* Study. *J Clin Med* 2023; 12(6):2434. [\[Crossref\]](#)
- Lima Nogueira BM, Da Costa Pereira TI, Pedrinha VF, de Almeida Rodrigues P. Effects of different irrigation solutions and protocols on mineral content and ultrastructure of root canal dentine. *Iran Endod J* 2018; 13(2):209.
- Botta SB, Ana PA, Santos MO, Zzell DM, Matos AB. Effect of dental tissue conditioners and matrix metalloproteinase inhibitors on type I collagen microstructure analyzed by Fourier transform infrared spectroscopy. *J Biomed Mater Res B Appl Biomater*. 2012; 100(4):1009–16. [\[Crossref\]](#)
- LeGeros RZ. Biological and synthetic apatites. In: *Hydroxyapatite and Related Compounds*, editors. Hydroxyapatite and Related Materials. 1st edition. USA. CRC press; 1994. p. 3–11. [\[Crossref\]](#)
- Kinney JH, Pople JA, Marshall GW, Marshall SJ. Collagen orientation and crystallite size in human dentin: A small angle X-ray scattering study. *Calcif Tissue Int* 2001; 69(1):31. [\[Crossref\]](#)
- Guo X, Miao H, Li L, Zhang S, Zhou D, Lu Y, et al. Efficacy of four different irrigation techniques combined with 60°C 3% sodium hypochlorite and 17% EDTA in smear layer removal. *BMC Oral Health*. 2014; 14:1–6. [\[Crossref\]](#)
- Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. *Int Endod J* 2009; 42(4):335–43. [\[Crossref\]](#)
- Wu L, Mu Y, Deng X, Zhang S, Zhou D. Comparison of the effect of four decalcifying agents combined with 60°C 3% sodium hypochlorite on smear layer removal. *J Endod* 2012; 38(3):381–84. [\[Crossref\]](#)
- Marques JA, Falacho RI, Santos JM, Ramos JC, Palma PJ. Effects of endodontic irrigation solutions on structural, chemical, and mechanical properties of coronal dentin: A scoping review. *J Esthet Restor Dent* 2024; 36(4):606–19. [\[Crossref\]](#)
- Liñan Fernández M, González Pérez G, Ortiz Villagómez M, Ortiz Villagómez G, Mondragón Báez TD, Guerrero Lara G. Estudio *In vitro* del grado de erosión que provoca el EDTA sobre la dentina del conducto radicular. *Rev Odontol Mex* 2012; 16(1):8–13. [\[Crossref\]](#)
- Calt S, Serper A. Time-Dependent Effects of EDTA on Dentin Structures. *J Endod* 2002; 28(1):17–9. [\[Crossref\]](#)
- Tay FR, Pashley DH, Loushine RJ, Doyle MD, Gillespie WT, Weller RN, et al. Ultrastructure of smear layer-covered intraradicular dentin after irrigation with BioPure MTAD. *J Endod* 2006; 32(3):218–21. [\[Crossref\]](#)
- Combes C, Cazalbou S, Rey C. Apatite biomaterials. *Minerals* 2016; 6(2):34. [\[Crossref\]](#)
- Beltz RE, Torabinejad M, Poursmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. *J Endod* 2003; 29(5):334–37. [\[Crossref\]](#)
- De-Deus G, Zehnder M, Reis C, Fidel S, Fidel RAS, Galan J, et al. Longitudinal Co-site Optical Microscopy Study on the Chelating Ability of Etidronate and EDTA Using a Comparative Single-tooth Model. *J Endod* 2008; 34(1):71–5. [\[Crossref\]](#)

34. Hatipoğlu Ö, Martins JFB, Karobari MI, Taha N, Aldhelai TA, Ayyad DM, et al. Clinical Decision-Making of Repair vs. Replacement of Defective Direct Dental Restorations: A Multinational Cross-Sectional Study With Meta-Analysis. *J Esthet Restor Dent* 2025 ;37(4):977–91. [\[Crossref\]](#)
35. Lehmann A, Nijakowski K, Jankowski J, Donnermeyer D, Palma PJ, Drobac M, et al. Awareness of possible complications associated with direct composite restorations: A multinational survey among dentists from 13 countries with meta-analysis. *J Dent*. 2024; 145:105009. [\[Crossref\]](#)