Comparative Evaluation of the Effect of Two Chelating Agents on Dentin-root Microhardness: An In-vitro Pilot Study

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Abstract

To compare the chelation effect on dentin-root microhardness and surface topography between 17% Ethylenediaminetetraacetic acid (EDTA), a commonly used chelating agent, and a potential chelating agent 25% copolymer of acrylic acid and maleic acid (Poly (AA-co-MA).

Eleven extracted, well-preserved maxillary incisors were used. After the routine procedure of endodontic instrumentation, the roots were longitudinally split, dividing them into two halves. The samples were randomly divided into three groups of seven each (n=7). Group one was subjected to 17% EDTA. Group two was subjected to 25% Poly (AA-co-MA). Group 3 was used as a control group and treated only with saline. The chelating agents and saline were applied in a standardized method. The microhardness was evaluated using the Vickers Hardness test which was applied in three different spots on every half tooth. The surface topography was evaluated under Scanning Electronic Microscope. Paired t-test and Post Hoc tests with analysis of variance (ANOVA) were used to estimate the impact of the chelating agents on the dentin-root microhardness and to compare the two agents.

A significant difference in the microhardness was observed within Group 1 and Group 2 (before/after) ($p<0.05$), but no significant difference was seen between Groups 1 and 2 ($p>0.05$). A scanning electron microscope revealed the clear impact of EDTA and Poly (AA-co-MA) in the removal of the smear layer compared with saline.

Within the limitations of this study, it is concluded that 25% Poly (AA-co-MA) can be used as a chelating agent. It has an equal effect to 17% Ethylenediaminetetraacetic acid (EDTA) in reducing the dentin-root microhardness.

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Introduction

Endodontic treatment is a popular procedure in deep caries exposing the pulp, necrotic pulp, or irreversible pulp inflammation or infection. The steps in root canal treatment include diagnosis, which confirms the need for endodontic therapy, followed by access cavity preparation, extirpation of the pulp tissue, debridement, irrigation, drying, obturating, and then placing the final restoration. The effect of endodontic treatment also affects the root canal geometry.[1] The chelating agent is used during irrigation of canals to remove the smear layer, increase dentine permeability and ease achieving a good apical seal. It also functions as a disinfectant and lubricant for ease of root-canal instrumentation.[2,3] In 1957, Nygaard-Ostby was one of the pioneers who reported using Ethylenediaminetetraacetic acid (EDTA) to clean, widen, and negotiate calcified canals.[4] Till now, 17% EDTA is one of the most commonly used chelators.[5,6]

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An ideal irrigant can possess a long-term antibacterial effect, be biocompatible, and remove the smear layer. The irrigant should not negatively affect the dentin by decreasing the restorative material's adhesiveness, enhancing microleakage, or activating endotoxins.[7,8]

Based on the mode of action, the irrigant can be an antibacterial or decalcifying agent. Some of the irrigants can have a combined effect. There are two types of chelating agents based on their consistency as liquid and paste types. Ostby reported that EDTA forms complex ions with calcium present in dentine and causes decalcification. He also observed that the depth of decalcification did not exceed 50 microns. Continuous attempts were reported to improve or adjust EDTA. EDTAC was a combination of EDTA and quaternary ammonium compound, which was added to reduce the surface tension of the irrigant and increase wetting.[9] However, the EDTA was more biocompatible and effective when combined with sodium hypochlorite. In a scanning electron microscopy study, the authors reported that the best outcome could be reached using the chelating agent (EDTA) after the irrigants: chlorhexidine gluconate or sodium hypochlorite.[10] Many other irrigants and chelating agents were tested and considered by the researcher as helpful materials for endodontic treatment; for example, citric acid, EGTA (ethylene glycol-bis(β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid), and tetracycline hydrochloride.[11,12] A solo use or a mixture of doxycycline, citric acid, and a detergent was also tested as an endodontic irrigant.[13, 14] This material possessed antibacterial properties but was required to be used along with sodium hypochlorite.[15] The ideal material is also expected to have antibacterial properties.[16]

Most chelating materials remove the smear layer in varying compositions and time applications and affect the dentin microhardness to different degrees.[17]

Maleic acid was used as a chelating agent, and it was found to increase surface roughness more than EDTA. However, it reduces the microhardness of dentin-root similarly to the EDTA.[18]

A recent study showed that poly(AA-co-MA) effectively removes the smear layer and debris. This copolymer is formed by radical copolymerization of maleic anhydride with acrylic comonomers. Görduysus et al. used poly(AA-co-MA) for endodontic purposes as an irrigant.[19]

They reported that there was no erosion when used alone as a chelating agent or when used along with a hypochlorite solution. Poly(AA-co-MA) was nominated to be used in medicine for its chelating properties.[20] Although it is considered one of the polymeric materials used in glass-ionomer cement, no study was found in the literature that covered the effect of the copolymer of acrylic and maleic acid on dentin microhardness when used as an irrigant for endodontic treatment. The main goal of this study was to evaluate the impact of the poly (AA-co-MA) agent on the microhardness of dentin-root and compare it with the standard chelating agent: EDTA.[21]

Materials and Methods

Eleven newly extracted, well-preserved (Thymol at 4 degrees), single-rooted anterior human teeth were used. Any tooth with an extensive defect/filling, caries, curved canals, or endo-treated was excluded. All the teeth are caries-free, with maxillary incisors with straight canals.

The teeth were decoronated at the cementoenamel junction using a high-speed bur. The roots were prepared to an apical file size of 30 (F3) with ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). Between each instrumentation, the canals were irrigated with 2 mL 2.5% Sodium hypochlorite (NaOCl) for 30 seconds for each flush. After this procedure, the roots were split into two equal pieces longitudinally. The initial grooves for splitting the teeth were placed vertically on the buccal and palatal surfaces using a water-cooled diamond wheel bur with a low-speed handpiece (200 rpm). The final through-and-through splitting was done in the buccopalatal direction using a chisel and mallet. The split pieces from each root were inspected for any defects. After a thorough inspection, they were mounted in self-cured acrylic resin with the dentin root exposed. (Figure1) The mounted specimens were polished with coarse to fine silicon carbide paper (1200-3000 grit) and finished with diamond paste. The specimens were randomly allocated to one of three groups (n=7 half teeth):

Group 1: 2 mL, 17% Ethylenediaminetetraacetic acid (EDTA) 1 min.

Group 2: 2 mL, 25% copolymer of acrylic acid

and maleic acid (Poly (AA-co-MA) 1 min.
Group 3: 2 mL, Saline (control) 1 min.

Figure 1. Mounted root sections in acrylic resin.

The sample size calculation was following similar previous in-vitro studies with a significance level set at 0.05 and a power of 80%.[22]

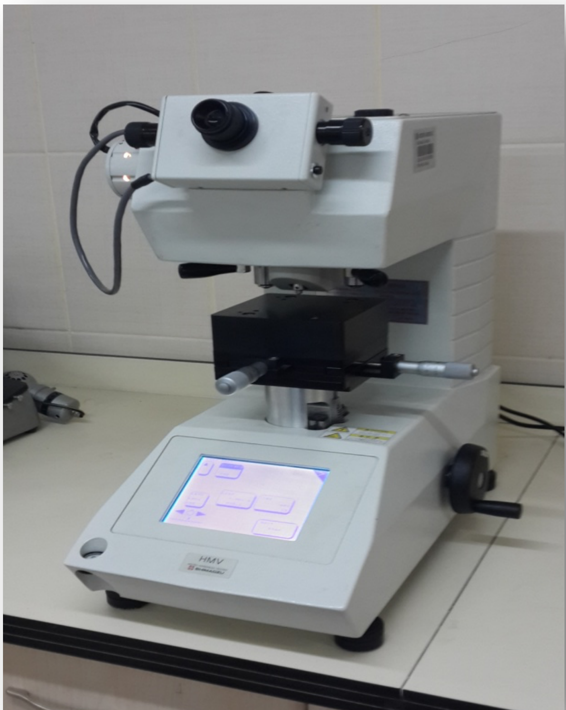

Figure 2. Vickers microhardness tester (HMV Microhardness tester, Shimadzu).

Microhardness measurement

A total volume of 1 ml of each solution was applied to each specimen for 1 minute with agitation using a micro brush. All specimens were cleaned with saline solution by the ultrasonic system and dried before measuring microhardness. Two sets of microhardness readings were recorded (before-after). The initial microhardness measurements were performed before using the chelating agent. A Vickers microhardness tester (HMV Microhardness tester, Shimadzu) with a load of 200g for 15 seconds was used (Figure 2). The locations were chosen parallel to the edge of the root canal lumen, at a depth of 100μm from the pulp–dentine interface. Three separate indentations were made for each specimen. These locations and indentations were observed under a stereomicroscope. The second set of readings was made after applying the chelating agent: 17% EDTA (Vista Dental Products, Racine, WI, USA) or 25% Poly (AA-co-MA) (Aldrich Chemical Co., Milwaukee, WI, USA). The application time for all the solutions was 1 minute. After immersion in the irrigant solutions, the specimens were rinsed immediately with distilled water to avoid the prolonged effects of chelating solutions. New indentations (after) were made on each specimen adjacent to the initial ones (before), and the microhardness values were recorded (Table 1). Finally, 7x3=21 readings before and 21 after were recorded for every group.

Scanning Electron Microscope (SEM) examination

Six randomly selected specimens (two from each group) representative of the surface micromorphology were examined using Scanning Electron Microscope (SEM) (JSM-6400, JEOL Ltd., Tokyo, Japan) at a magnification of x1000. The removal of the smear layer after using the chelating agents was compared with the saline control group image (Figure 3).

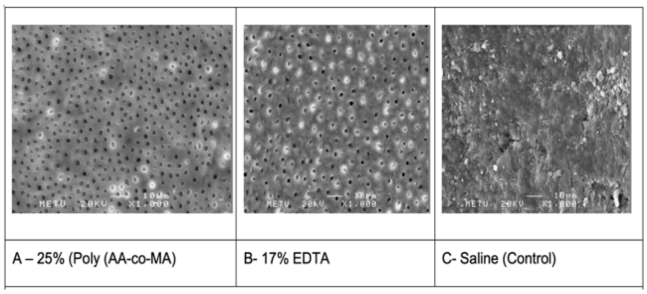

Figure 3. Scanning Electron Microscope: Bar = 10 µm; original magnification, x1000. Photomicrographs showed a clear impact of 25% EDTA (A) and 17% Poly (AA-co-MA) (B) in the removal of the smear layer compared with saline (C). Debris and heavy smear layer can be easily detected in (C) (Saline/Control).

Statistical analysis

Data were entered into the Statistical Package for Social Science software(SPSS, version 26). Both descriptive and inferential statistics were used to describe the sample and identify differences in the sample. The exact (and approximate) 95% confidence intervals, test statistics, and p-values were reported. The p-value ($p < 0.05$) was defined as statistically significant.

Descriptive statistics, the Kolmogorov-Smirnova test, and the Shapiro test were used to evaluate the data and check the normality of the sample, to guide the analysis to use parametric or non-parametric tests. The paired t-test was used to estimate the differences before and after applying the chelating agents. One-way analysis of variance (ANOVA) and Post hoc determined whether there were any significant differences between groups.

Results

The results revealed a decrease in microhardness after using the chelating agents. The summary of microhardness before and after treatment with the amount of change in the tested materials can be reviewed in Table 1.

Summary of dentin-root microhardness before and after the use of EDTA.					
	N	Minimum	Maximum	Mean	Std. Deviation
Before	21	48.00	70.60	55.90	6.10
After	21	35.40	70.40	49.58	9.29
Changes	21	-12.40	29.40	6.32	10.75
Percent Changes	21	-22.92	44.77	10.53	17.57
Summary of dentin-root microhardness before and after the use of Copolymer of Acrylic Acid and Maleic Acid					
	N	Minimum	Maximum	Mean	Std. Deviation
Before	21	43.70	80.80	65.57	9.45
After	21	45.90	73.50	58.17	8.47
Changes	21	-22.60	31.70	7.40	13.70
Percent Changes	21	-51.72	40.85	8.87	22.241

Table 1. Summary of dentin-root microhardness before and after treatment.

	Kolmogorov-Smirnov^a			Shapiro-Wilk		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Changes	.065	63	.200*	.989	63	.861

Table 2. Tests of Normality.

Shapiro-Wilk test and Kolmogorov-Smirnova test were used to check the normality of data to decide whether to use a parametric or non-parametric test. The result showed that the data is normally distributed as a p-value >0.05, Table 2. The mean with the standard deviation was used to summarize the change after treatment.

Paired t-test was used to find the mean difference before and after treatment. The result showed a statistically significant decrease in dentin-root microhardness when 17% EDTA was used. The microhardness means decreased significantly from 55.90±1.33 (before) to 49.58±9.29 (after), $p < 0.05$. A similar result was noticed when a 25% Copolymer of Acrylic Acid and Maleic Acid was applied. There was a statistically significant decrease in the microhardness from 65.57±9.45 (before) to 58.17±8.47 (after).

In the one-way ANOVA test, the readings achieved 81% power to detect differences among the means versus the alternative of equal means using an F test with a 0.05 significance level. The copolymer of Acrylic Acid and Maleic Acid was able to reduce the microhardness more than EDTA. However, the difference was not statistically significant. One-way Analysis of Variance (ANOVA) did not detect a significant difference between the two chelating agents.

Discussion

The main goal of this study was to evaluate the effect of the 25% poly (AA-co-MA) chelating agent and its impact on the microhardness of dentin-root and to compare it with the standard chelating agent 17% EDTA. 25% poly (AA-co-MA) and 17% EDTA can reduce the dentine-root microhardness significantly. The study was not able to detect a significant difference between the two chelating agents. Chelating agents are used in root canal treatments to remove the smear layer The scanning electron microscope images revealed that 25% poly (AA-co-MA) and 17% EDTA are effective in removing the smear layer compared to the control group (Saline). Apart from the removal of the smear layer, most chelating agents demineralize and soften the dentin root. Though softening of the root is beneficial in clinical cases where calcified root canals are present, it can change the characteristics of dentin-root properties. [2,7] In normal root morphology and calcified canals, dentin softening aids in easy access to the root canal system. However, altering the microhardness of the dentin root may affect the bonding of the restorative materials.[23] Literature evidence suggests that dentin root is softened when chelating agents are applied.[24,25] Moreover, there is a growing body of literature that reported an increase in dentin erosion with various combinations and types of chelating agents such as citric acid, EGTA (ethylene glycol bis tetraacetic acid), EDTAC (EDTA with Cetavlon), DTPA (diethyl-triamine-pentaacetic acid), and MTAD (a mixture of a tetracycline isomer, an acid, and a detergent).[26,27]

The application time of irrigants is crucial in reaching a good result. De Deus et al. evaluated three different irrigants, EDTA, EDTAC, and citric acid, with varying application times. He reported that an application time of three minutes for EDTA and EDTAC was apt to reach a significant difference between the two materials in reducing the dentin microhardness. After five minutes, this difference will become minimal and insignificant.[28].In another study by Calt et al., a one-minute application of 17% EDTA was sufficient to remove the smear layer. Removal of the smear layer is essential to prevent bacterial invasion in root-treated teeth. Calt et al. also recommended avoiding the application of 17% EDTA for longer than one minute.[29] It was observed by many authors that extended application times of EDTA increase the removal of the calcium from the dentin root. This results in excessive tubular erosion.[30]

Therefore, a prolonged application of the chelating agents (>1 min) was avoided in the current study. Sayin et al. compared EDTA, EGTA, EDTAC, tetracycline HCL, and sodium hypochlorite. They noted that the radicular dentin softened after irrigant application and the softening of radicular dentin was different in coronal, middle, and apical areas. Their observations demonstrated that tetracycline HCL and Sodium hypochlorite (NAOCL) had the least impact on radicular dentine and the EDTA, when combined with sodium hypochlorite, had the maximum decrease in dentin-root microhardness.[27] Ballal et al. compared 17% EDTA with 7% maleic acid. Both EDTA and maleic acid (both were applied for one minute) had the same effects on microhardness, but maleic acid had more surface roughness when compared to EDTA.[18]A previous study compared the impact of 17% EDTA, 17% EDTAC, and 10% citric acid on the dentin-root microhardness.[28] No substantial difference between EDTA and EDTAC was reported after one minute. The study also showed that the weakest material was citric acid. However, in another study, increasing the concentration of citric acid to 19% make it more effective in decreasing the microhardness than 17% EDTA (both are followed by 5.25% NaOCl).[31]

The probable reason for these differences could be the difference in the loss of dentin calcium after applying the chelating agent.[32] The current study proved that combining maleic acid with acrylic acid produces an effective chelating agent. It was reported that maleic acid and its copolymers, due to chelating capabilities, were used for biomedical purposes. They are evidence-based medicine that supports using maleic copolymers as dental materials, drugs, enzyme-conjugates, or gene delivery systems.[21] Görduysus et al. compared smear layer removal between Poly(AA-co-MA) and EDTA. They concluded that poly(AA-co-MA) could remove the smear layer effectively and have better surface roughness, which may increase the adhesion of restorative materials.[19] In another study, Ulusoy et al. reported that using 2.5% sodium hypochlorite (NaOCL) with maleic acid demonstrated a more effective elimination of the smear layer than 17% EDTA and NaOCL, 1.3% NaOCl + MTAD, or 5% NaOCl.[33] Kouvas et al. showed that removing the smear layer enables sealer penetration and gives a proper seal to root-treated teeth. They confirmed their finding by scanning electron microscope images.[8]

Scanning electron microscopy (SEM) images were performed in the current study to report the impact of the tested materials on the smear layer. The SEM images allow for clear visualization of the smear layer removal. Many authors estimated the mineral loss in dentin by measuring the microhardness and used the SEM to support their findings.[34,35,36]

In the current study, the SEM images showed smear-free surfaces after the use of EDTA and poly (AA-co-MA). The peritubular and intertubular appeared smooth and flat after 1 min application of the chelating agents, Figure 3. The SEM images can't be considered the primary data set of this study. Instead, the images support the microhardness test results and must be interpreted with caution. Some of the limitations of the present study is generalizing the results to clinical scenarios requires more in vivo and in vitro investigations with bigger sample sizes and diverse clinical settings.

Conclusions

Within the limitations of this study, it is concluded that a 25% copolymer of acrylic acid and maleic acid (Poly (AA-co-MA)) can be used as an effective chelating agent. It has an equal effect to 17% Ethylenediaminetetraacetic acid (EDTA) in reducing the dentin-root microhardness. More studies are needed to generalize the novel usage of the Poly (AA-co-MA) as an endodontic chelating agent.

Declaration of Interest

The authors report no conflict of interest.

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