

Polymer-Based Chemistry of Carbohydrate-Derived Fulvic Acid and Its Comparative Effect with Chelating Agents on Root Dentin Microhardness

Roshan M. Samuel^{1,*}, Rushikesh Mahaparale², Priyanka Zinge³

Abstract

The interaction between the polymer science and the endodontic biomaterials field has increased recently while considering the search for chelating agents. CHD-FA (Carbohydrate-Derived Fulvic Acid), a naturally occurring low-molecular-weight polymeric organic acid, has potential chelating and antimicrobial and antioxidant activities. This investigation examines the polymeric chemistry of CHD-FA and compares the effect of CHD-FA with other chelating agents, ethylenediaminetetraacetic acid (EDTA), and citric acid, on root dentin microhardness. A total of thirty human extracted mandibular premolars were decoronated, instrumented, and irrigated with 5% CHD-FA, 17% EDTA, and 20% citric acid, and hardness was measured with a Vickers indenter at the apical third. It was statistically deduced that CHD-FA was the only agent that was better at preserving dentin microhardness than EDTA and citric acid, as the latter two caused a greater loss of softer dentin and demineralization. With the unique molecular architecture of CHD-FA and the greater presence of carboxyl, ester, and amide groups, complexation of calcium was manipulated with little to no loss of dentin matrix structural integrity. Additionally, compared to EDTA, CHD-FA's polymeric functional groups provide a means for controlled chelation, thus less dentin erosion is resulted. Combined with the microbicidal and oxidative activities, CHD-FA correlates to a grand major constituent endodontic irrigant. More than that, the research also demonstrates the importance of polymer chemistry in creating other bio-based chelators with better effectiveness and compatibility. CHD-FA has been an innovatively sustainable option which is polymer-derived as well. It has also offered a unique ability to remove smear layers while evenly preserving dentin. This study advances the polymer-based fulvic acid formulations in endodontics and extends the range of use of polymer composites in dental biomaterials.

Keywords: Polymer chemistry; carbohydrate-derived fulvic acid; chelating agents; root dentin microhardness; EDTA; citric acid; bio-based polymers; dental composites

*Author for Correspondence

Roshan M. Samuel

¹Professor & HOD, Department of Conservative Dentistry & Endodontics, School of Dental Sciences, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

²Professor, Department of Conservative Dentistry & Endodontics, School of Dental Sciences, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

³Associate Professor, Department of Conservative Dentistry & Endodontics, School of Dental Sciences, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, India

Received Date: October 14, 2025

Accepted Date: December 04, 2025

Published Date: April 27, 2026

Citation: Roshan M. Samuel, Rushikesh Mahaparale, Priyanka Zinge. Polymer-Based Chemistry of Carbohydrate-Derived Fulvic Acid and Its Comparative Effect with Chelating Agents on Root Dentin Microhardness. Journal of Polymer & Composites. 2026; 14(Special Issue 2): S1051–S1061p.

INTRODUCTION

The combination of polymer science and endodontic biomaterials has opened new opportunities for substitution of biocompatible and effective candidates for traditional chelators. CHD-FA is a low-molecular-weight polymeric organic acid of natural origin, which exhibits chelating, antimicrobial, and antioxidant activity. Successful endodontic therapy requires the removal of bacteria and infected dentin, which means getting straight-line access to the root canals, thorough debridement by means of careful biomechanical preparation, effective disinfection, and then full obturation of the root canal system. This process blocks all possible paths of communication between the

pulpal and peri-radicular tissues. In addition to optimal cleaning and sealing of the root canal system to prevent bacterial infiltration, which is one of the causes of post-treatment complications, it also eliminates factors that may cause recontamination or reinfection. [1] During root canal cleaning and shaping procedures with hand or rotary instruments, there is a tendency for the remaining organic pulp tissues and inorganic dentinal debris to accumulate on the canal walls. [2] Mokhtari H et.al, first described the presence of thin, sludge-like mass which is now referred to as the “smear layer”, covering the surfaces of the canals that had received instrumentation. [3] This layer is made up of dentin particles, tissue debris, microorganisms, and their metabolic by-products.

The smear layer normally consists of particles that are approximately 0.5 μ m in diameter as well as about 15 μ m with an average thickness of between 1 to 5 μ m.[4] Ballullaya SV et al. posited that this layer negatively impacted both apical and coronal sealing by causing microleakage that reduced the durability of endodontic therapy. Therefore, complete removal of the organic and inorganic debris is necessary prior to the obturation of the root canal system. [5] There are several agents that are available and are regularly used for smear layer removal. Sodium hypochlorite is an example of a solution that is able to dissolve smear layer. It is well known that NaOCl dissolves organic tissues [6] and with increasing temperature, the effect is intensified.[7] There is also chelate solution like EDTA. EDTA is a standard chelating agent that forms soluble calcium chelates by reacting with calcium found in dentin. Reports say that EDTA can decalcify dentine to a depth of about 20–30 μ m within five minutes.[8] Conversely, root dentin is severely demineralized when EDTA is used.

Consequently, EDTA also weakens root dentin and decreases its fracture resistance. In periapical tissue, cytotoxic effects occur when EDTA leaks and weak antibacterial effects are noted.[9][10] Demineralizing agents, including chitosan, citric acid, maleic acid, and apple cider vinegar, are employed due to their chelation, low toxicity, and anti-microbial effects.[11] Nevertheless, the search continues for natural irrigants that are biocompatible and cause less adverse effects to the dentin microstructure. Humins, humic acid and fulvic acid are natural decomposition products of organic matter, specifically decayed residues of plants and animals. Fulvic acid (FA), one of the “humic” compounds, is the only one that remains fully dissolved in water at all pH levels. FA is produced when humic acid is purified, for example, during a wet oxidation process with carbohydrates such as glucose, sucrose, and starch as well as cellulose (CHD-FA is low molecular weight, pale, and less concentrated, SA Patent 2001/2419). Without comparison to FA, CHD-FA is a harmless organic acid for medical and pharmaceutical applications as it has no toxic heavy metals.

It has antiviral, antifungal, antibacterial, chelating, anti-inflammatory, and antioxidant properties. It is also low-molecular-weight, cationic and heat-stable. The relative concentrations of the molecules which comprise them are higher than usual, namely, the carboxyl, ester, amide, and aliphatic carbon groups. [12] The purpose of this in vitro research was to evaluate the effects of using 5% CHD-FA, 17% EDTA, and 20% citric acid as final irrigating solutions on the microhardness of root dentin. This investigation was specifically directed to the constituent CHD-FA of the dentin and microhardness as well as the other standard chelating solutions, citric acid, and ethylenediaminetetraacetic acid (EDTA).

MATERIALS AND METHODS

A power analysis conducted using G Power (version 3.0.1) indicated that a minimum of 10 specimens per group would provide 80% statistical power to detect significant differences, assuming an effect size of 0.9 and a significance level of 0.05. Therefore, the final sample size was set at thirty specimens per group. For the study, a total of thirty human mandibular single canal premolars with mature roots that radiographically confirmed were removed for periodontal and orthodontic procedures. The study protocol was approved by the Institutional Review Board and Ethics Committee (Approval No. SRMDC/IRB/2017/MDS/No.301). Teeth having caries, fracture, calcified canals, morphological defects, and significant root curvature were not included. To keep the teeth hydrated until they were used, they were washed and kept in a 0.1% thymol solution at 37°C. (Figure 1)



Figure 1. Sample size.



Figure 2. 15 mm root length was marked.



Figure 3. Decoronated samples.

A 10 size K-file that was passed through the apical foramen was used to determine the canal's patency. (Figure 4) The size of the working length was found to be 1 mm less than the size of the size 15 K-file that was just leaving the foramen.

The CHD-FA (Mineralife Nutraceuticals, USA) used in this study was prepared by diluting it with distilled water to achieve a 5% concentration. The crowns of the teeth were removed using a high-speed diamond disc (Axis Dental Corp., Switzerland) under continuous water cooling to standardize the root length at 15 mm (Figures. 2 and 3).

To simulate the vapor lock effect, the root apices were sealed with sticky wax (Figure 5). Each root canal was then prepared in a crown-down technique using Dentsply Protaper rotary files up to F3 (Figure 6).

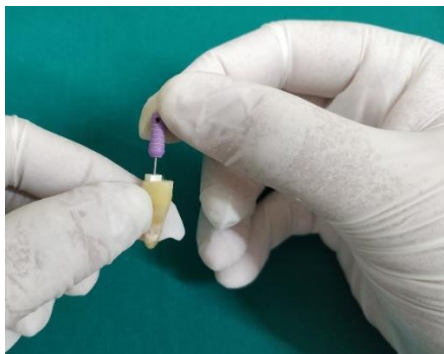


Figure 4. Canal patency was established using 10 k file.



Figure 5. Root apices sealed with sticky wax.



Figure 6. Biomechanical preparation was done till F3 universal rotary protaper.



Figure 7. Irrigation with 5% fulvic acid.

Three groups were randomly selected from among all the samples.

Group 1 (CHD-FA; n=10)

After using each instrument, rinse it for one minute with 5 mL of 5.25% NaOCl and then rinse it one last time with 5 mL of 5% CHD-FA. After one minute of active irrigation, a 5-milliliter distilled water rinse was employed. (Figure 7)

Group 2 (EDTA; n=10)

After using each instrument, rinse it for one minute with 5 mL of 5.25% NaOCl and then rinse it one last time with 5 mL of 17% EDTA. After one minute of active irrigation, a 5-milliliter distilled water rinse was employed. (Figure 8)

Group 3 (Citric acid; n=10)

After using each instrument, rinse it for one minute with 5 mL of 5.25% NaOCl and then rinse it one last time with 5 mL of 20% citric acid. After one minute of active irrigation, a 5-milliliter distilled water rinse was employed. (Figure 9)

Absorbent paper points were used to dry the canals, and cotton pellets were placed to protect the canal orifices (Figure 10). To facilitate splitting the roots into two equal halves, two longitudinal grooves were created on the external surface using a diamond disc at low speed under water coolant, ensuring the canal was not perforated (Figure 11). The teeth were then split using a chisel.



Figure 8. Irrigation with 17% EDTA.



Figure 9. Irrigation with 20% citric acid.

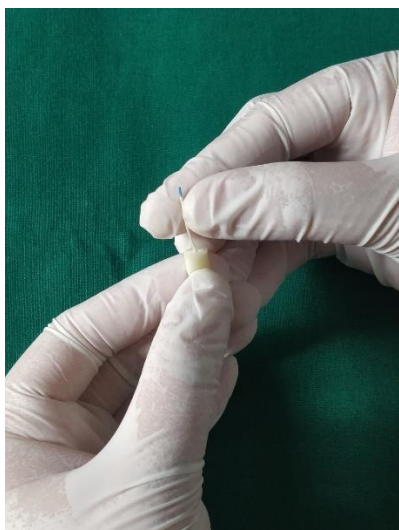


Figure 10. Canals were dried with absorbent paper points.

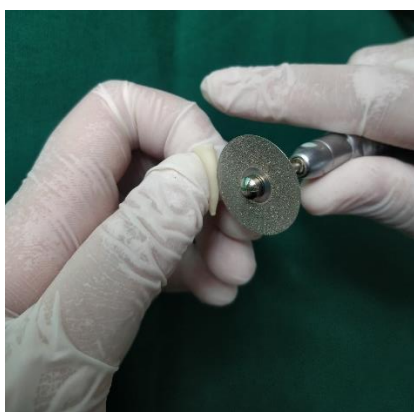


Figure 11. Longitudinal grooves were made with diamond disk.

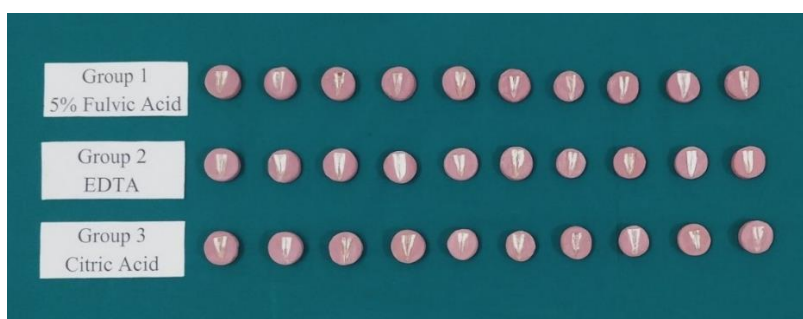


Figure 12. The samples were mounted in acrylic resin.

Each root sample was split in half and placed horizontally in auto polymerizing acrylic resin, exposing the canal lumen. (Figure 12) After removing any surface defects with 1500 grit fine emery paper under distilled water, the mounted samples were polished using a felt cloth that had been treated with 0.1 μm alumina particles.

Using a Vickers diamond indenter with a 300 g load and a dwell time of 20 seconds, three indentations were made on the apical third of the root dentin (4 mm from the apex) (Figure 13). The Vickers hardness number (VHN) for each sample was determined by calculating the average of these three measurements.

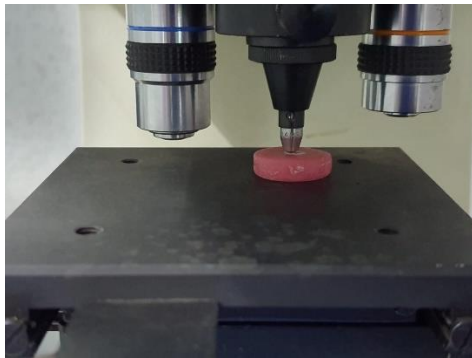


Figure 13. Indentations were made using a Vickers microhardness tester.

Statistical Analysis

Data were analyzed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). Intergroup variations in microhardness values were assessed through a one-way ANOVA (F-test) with a significance threshold of 0.05. Tukey's post-hoc test was subsequently employed for multiple pairwise comparisons.

RESULTS

Figure 14 illustrates the mean values obtained for each group, while Table 1 presents the summarized Vickers Hardness Number (VHN) values (mean \pm SD). Among the tested groups, Group 1 showed the highest average VHN, followed by Groups 2 and 3. Statistical analysis revealed a highly significant variation among the groups ($P < 0.001$). Further pairwise evaluation demonstrated significant differences between Group 1 and Group 2 ($P < 0.001$) as well as between Group 1 and Group 3 ($P < 0.001$). However, the difference between Group 2 and Group 3 was not statistically significant ($P = 0.024$). Figure 15 shows the Pairwise comparative statistics of different chelating agents for root dentin microhardness using Tukey's post hoc test.

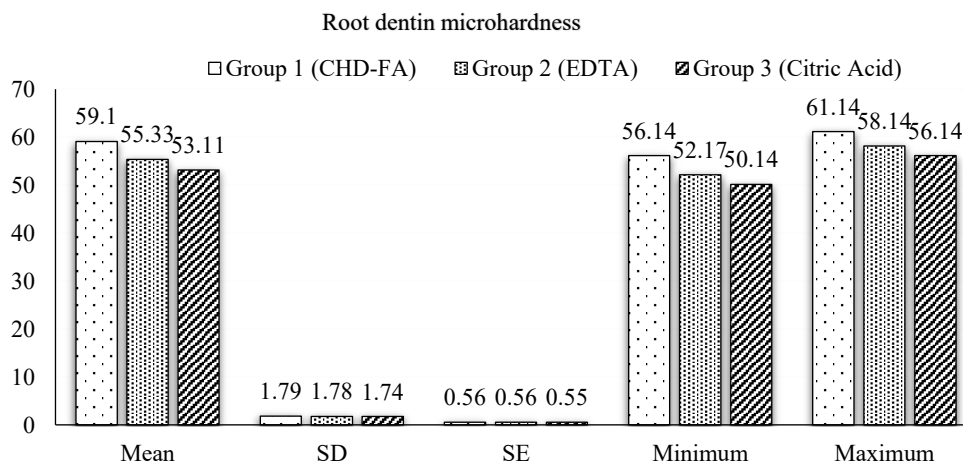


Figure 14. Graphical presentation of different chelating agents for root dentin microhardness.

Table 1. Vickers microhardness values (Mean \pm Standard deviation) of root canal dentin of different groups.

Groups	Mean \pm standard deviation
CHD- FA	59.1 \pm 1.79
EDTA	55.33 \pm 1.78
Citric acid	53.11 \pm 1.74

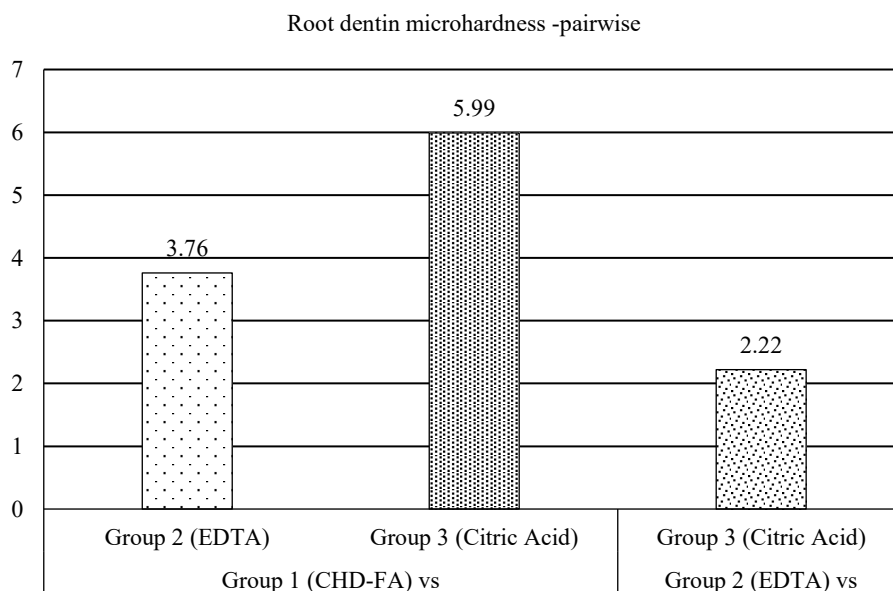


Figure 15. Pairwise comparative statistics of different chelating agents for root dentin microhardness using Tukey's post hoc test.

DISCUSSION

The research focused on determining the effects that 5% CHD-FA, 17% EDTA, and 20% citric acid have on root dentin microhardness to ascertain if a naturally derived chelating agent may be more effective in preserving dentin structure compared to the traditionally used chelating solutions. Results demonstrated a statistically significant difference between the three groups with CHD-FA having the most dentin microhardness, and EDTA and citric acid had the least. [13, 14] These findings play an important role in clinical practice as the dentin microhardness must be protected as it is vital to the structure, fracture resistance, and long-term stability of the root canal-treated tooth. Chelating solutions demineralize dentin, and while some alteration is intended due to smear-layer removal, demineralizing dentin excessively is detrimental to the dentin's mechanical properties.[15] In the field of endodontics, chelation has for many years relied on EDTA, which has a notorious reputation for its rampant decalcification.

By binding to calcium ions within dentin hydroxyapatite, this results in a considerable loss of minerals and a change in the calcium-phosphorus ratio, thus weakening the dentin matrix [16]. Long-term exposure has been correlated to erosion and widening of the dentinal tubules, and loss of microhardness, all of which would negatively influence the adhesion of sealers and the prognosis of the examined tooth [17, 18]. Citric acid, another chelating agent, is similarly able to soften and erode dentin and as the current study results citric acid has the greatest reduction in microhardness as compared to CHD-FA, thus corroborating those references. EDTA and citric acid, the chelating agents in question, prolonged exposure results in extreme weary microhardness. Both chelating agents present an erosion profile which is likely to instigate a compromise on the dentin mechanical properties, thus losing the purpose of a final rinse on preserving any dentin. While CHD-FA on the contrary has a more overall softer and milder effect compared to all other agents used on dentin microhardness.

The presence of carboxyl, ester, and amide functional groups in its structure makes it possible to control chelation without excessive demineralization [12]. Unlike EDTA, which strongly chelates calcium, CHD-FA forms rather weak complexes and is more dependent on the pH level of the solution. Additionally, the small size of the fulvic acid molecules facilitates its penetration into the apical third, which is the region where EDTA does not work well because of its high surface tension and limited fluid movement [19,20]. The result is a moderate chelating effect of CHD-FA, which explains the removal of smear layers without significant weakening of the substrate and the resulting higher

microhardness in this group. The results also underscore significant anatomical factors. The apical third of the root canal system is especially challenging because of the smaller canal diameter, its greater sclerosis, irregular deposition of dentin, and difficulty in the exchange of irrigants. These all affect the microhardness values and the action of the irrigants [21]. Because some chelators have decreased penetration and chelating effectiveness in this region, an irrigant like CHD-FA, with smaller molecules and moderate chelation, can improve the preservation of dentin structure. From a clinical perspective, the preservation of dentin microhardness is crucial for the longevity of endodontically treated teeth.

Excessive softening may predispose roots to vertical fractures, particularly in teeth with thin dentin walls. The integrity of the dentin also has importance for the bonding strength of resin-based sealers. Previous studies have documented the erosion of the strong chelators which diminishes the mechanical interlocking of the adhesive materials and therefore may compromise the sealing ability with greater microleakage [22]. The effect of CHD-FA in reduction of dentin degradation may improve predictability of obturation and long-term sealing efficacy. CHD-FA also has other chelating, antimicrobial, antioxidant, antiviral and anti-inflammatory activities which may be due to its special molecular structure [23]. These properties probably increase its value as an endodontic irrigating solution. An irrigating solution with the ability to profoundly decrease the microbial load, oxidative damage, and also conserve the dentin structure, would have a great clinical value, without the cytotoxic effects of EDTA due to its extrusion into periapical tissues [10]. The study also has limitations. The only microhardness was evaluated, and other possible variables such as dentin erosion depth, surface roughness, elemental composition, and bonding strength of the sealer were not evaluated.[24] Activation methods of irrigation which can significantly change the chelators efficacy were not used.

The in vitro environment of this study also cannot replicate key variables present clinically such as fluctuations in temperature, movement of fluids, and microbial diversity. Nonetheless, investigating different concentrations of CHD-FA in tandem with ultrasound or laser-activated systems, as well as the residual dentin's mechanical properties after exposure, would be valuable. Additional studies to measure the effect of CHD-FA on sealer penetration, bond strength, and vertical root fracture resistance would elucidate its clinical potential. Other clinical studies would also be needed to determine the compound's clinical safety and efficacy.[25] Overall, this study proposes CHD-FA to be a useful adjunct to classic chelating agents due to good smear layer removal, less microhardness dentin loss, and its chelating properties being well balanced. These characteristics are likely to facilitate the compound being incorporated into advanced flushing systems.

CONCLUSION

This research explains the importance of biocompatibility and the efficiency of chelating agents in endodontics. Carbohydrate-derived fulvic acid (CHD-FA), whose polymeric molecular structure is rich in carboxyl, ester and amide groups, preserves root dentin microhardness better than default agents do, especially EDTA and citric acid. CHD-FA provided controlled chelation and decreased erosion of dentin, making it a candidate as a sustainable alternative. Its chelating and other functions makes CHD-FA a multi-functional endodontic irrigant, and exemplifying the combination of polymer chemistry with clinical endodontics. CHD-FA has the potential to be the first of its kind, a bio-based polymer that can remove the smear layer while maintaining dentin integrity. This demonstrates the phylogenetic of polymer composites used in the dentistry field, specifically with the creation of endodontic irrigants that have therapeutic functions without the negative impacts that other molecules have. This research will validate the Durham et al sustainable polymer CHD-FA hypothesis with its long clinical testing, determining the most effective concentration, and integrating it with modern delivery systems to increase the effectiveness of CHD-FA. To sum up, this work highlights and acknowledges the significance of polymer chemistry in the improvements made in the field of endodontic materials and signifies the opportunities for the improvements of endodontics to include low risk, multifunctional and biofriendly alternative to the classical chelating agents. Based on the findings from this research, it can also be concluded that CHD-FA can be considered as a polymeric material that can be used in modern endodontic procedures and has a useful potential.

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