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Effect of Chelating Agents on Root Dentin Demineralization With or Without Diode Laser Activation: An In Vitro SEM–EDX Study

Vathsala N[1] Bhagavathi B C[2] Ashwija Shetty [3] Farhat Nasreen[4] KeerthiSagar[5] Keerthana M N[6]

[1]*Professor Department of Conservative Dentistry and Endodontics, The Oxford Dental College, Bengaluru, Karnataka, India*
[2]*Postgraduate Student, Department of Conservative Dentistry and Endodontics, The Oxford Dental College, Bengaluru, Karnataka, India*

Abstract:
Background: Chelating irrigants facilitate smear-layer removal but may alter the mineral phase of root dentin. Etidronic acid (HEDP) permits continuous chelation with sodium hypochlorite (NaOCl), while diode-laser activation may modify irrigant–dentin interaction. Aim: To compare the elemental surface composition of apical root dentin after 17% ethylenediaminetetraacetic acid (EDTA) or HEDP used with or without 980-nm diode-laser activation. Materials and Methods: Sixty extracted single-rooted mandibular first premolars were allocated to four groups (n=15): 17% EDTA without laser, 17% EDTA with laser, HEDP (Twin Kleen mixed with 3% NaOCl) without laser, and HEDP with laser. Laser activation used a 200-µm fiber, nominal wavelength 980 nm, maximum power 7 W, and a 20-s activation cycle. The apical dentin surface was examined by scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM–EDX). Oxygen (O), fluorine (F), phosphorus (P), calcium (Ca), and Ca/P mass ratio were analyzed. Kruskal–Wallis testing followed by Dunn's post hoc comparisons with Bonferroni correction was used (α=0.05). Results: Treatment significantly affected O (p<0.001), Ca (p<0.001), P (p=0.021), and Ca/P ratio (p<0.001), whereas F did not differ significantly (p=0.287). Calcium was highest in the HEDP-plus-laser group (40.80±2.82%), while the Ca/P ratio was highest in the laser-activated EDTA (2.54±0.15) and HEDP (2.51±0.22) groups. Conclusion: Both chelator type and laser activation influenced the elemental profile of apical root dentin. Laser-activated groups showed greater surface mineral exposure, while HEDP with laser produced the highest mean calcium content. These EDX findings should be interpreted as surface-compositional changes rather than direct measurement of bulk dentin mineral loss.

Keywords: *Diode laser; EDTA; energy-dispersive X-ray spectroscopy; etidronic acid; HEDP; root dentin; smear layer.*

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I. INTRODUCTION

Mechanical instrumentation produces a smear layer composed of inorganic dentinal particles and organic remnants. Although its clinical significance has been debated, the layer can impede irrigant contact with canal walls and interfere with penetration of sealers into dentinal tubules [1]. Chemical irrigation is therefore used to complement instrumentation, particularly in areas that instruments do not contact.

EDTA remains a widely used strong chelator for removing the inorganic component of the smear layer. Its chelation of calcium can, however, alter dentin surface chemistry and the Ca/P balance. SEM–EDS studies have demonstrated measurable changes in calcium, phosphorus and other elemental components after exposure to EDTA and alternative chelators [2–4].

Etidronic acid (1-hydroxyethane-1,1-diphosphonic acid; HEDP/HEBP) is a weaker chelator that can be combined with NaOCl, allowing a continuous-chelation strategy during instrumentation. Experimental work has shown that NaOCl/HEDP protocols can preserve more mineral than sequential NaOCl/EDTA protocols, reduce smear-layer accumulation, and produce less dentin erosion in some models [2,5–9]. Recent systematic reviews nevertheless emphasize protocol heterogeneity and the predominantly in-vitro nature of the evidence [10,11].

Irrigant activation is intended to improve solution exchange and contact with anatomically inaccessible areas. Sonic activation has enhanced the performance of continuous HEDP irrigation [6], and diode lasers have been investigated as an adjunct to EDTA and other irrigants for smear-layer modification [12–14]. A recent

SEM study specifically reported improved apical smear-layer removal when a 980-nm diode laser was combined with Twin Kleen [15].

Because effective smear-layer removal must be balanced against undesirable dentin alteration, simultaneous assessment of surface morphology and elemental composition is relevant. EDX permits quantitative characterization of the mineral-associated elements present on the analyzed dentin surface and has been used to compare chelators and activation protocols [3,4,16,17]. The present study therefore evaluated the effect of 17% EDTA and HEDP, with or without diode-laser activation, on the elemental surface composition of apical root dentin.

Aim and null hypothesis

The aim was to compare the elemental surface composition of root dentin after irrigation with 17% EDTA or HEDP (Twin Kleen mixed with 3% NaOCl), used with or without 980-nm diode-laser activation. The null hypothesis was that no significant differences would occur among the four groups in O, F, P, Ca, or Ca/P mass ratio.

II. MATERIALS AND METHODS

Study design and sample size

This in-vitro experimental study used 60 extracted human mandibular first premolars with a single root, single canal and closed apex. The planned sample size was 60 specimens (15 per group), based on an a priori calculation with $\alpha=0.05$ and 80% power. Teeth with previous endodontic treatment, calcification, resorption, immature apices, restorations, morphological defects, cracks or fracture lines were excluded.

Specimen preparation and canal instrumentation

The teeth were decoronated to obtain a standardized root length of 16 mm. Working length was established with a size 15 K-file. Root canals were prepared with Neoendo Neoblue rotary instruments according to the manufacturer's sequence. During preparation, 3% NaOCl was used between instruments, followed by saline. Irrigants were delivered with a 27-gauge needle. The apical foramina were externally sealed during irrigation to limit extrusion.

Experimental groups

Group	n	Final irrigation protocol
Group 1	15	2 mL of 17% EDTA for 1 min; no laser activation
Group 2	15	2 mL of 17% EDTA for 1 min followed by diode-laser activation
Group 3	15	2 mL of HEDP (Twin Kleen mixed with 3% NaOCl) for 1 min; no laser activation
Group 4	15	2 mL of HEDP (Twin Kleen mixed with 3% NaOCl) for 1 min followed by diode-laser activation

Diode-laser activation

For Groups 2 and 4, a diode laser was used with a 200-µm fiberoptic tip at a nominal wavelength of 980 nm and maximum power setting of 7 W. The tip was introduced into the canal and activated during gentle coronal withdrawal with a helicoidal movement, with re-entry toward the apical region, for a total activation cycle of 20 s. A final saline rinse was performed and canals were dried with absorbent paper points.

SEM–EDX evaluation

Longitudinal grooves were created on the external root surfaces and the roots were split. One half of each specimen was selected for analysis. Surface morphology was evaluated using scanning electron microscopy (JEOL JCM 6000Plus) with a secondary-electron detector in high-vacuum mode at approximately 500×–1500× magnification. Energy-dispersive X-ray spectroscopy was used to quantify the mass percentages of O, F, P and Ca on the apical root-canal dentin surface. The Ca/P mass ratio was calculated for each specimen as a composite descriptor of the analyzed mineral phase.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 26.0. Elemental mass percentages and Ca/P ratio were summarized using mean, standard deviation, median and range. Although Shapiro–Wilk testing suggested approximate within-group normality, variance heterogeneity was present for several outcomes; therefore, Kruskal–Wallis tests were used for four-group comparisons. Significant omnibus tests were followed by Dunn's pairwise comparisons with Bonferroni correction. Epsilon-squared (ε^2) was used as the omnibus effect-size measure. Statistical significance was set at $p<0.05$.

III. RESULTS

The four irrigation protocols produced distinct elemental surface profiles in the apical third. Significant overall group differences were detected for O, Ca, P and Ca/P ratio, while F showed no significant group effect.

Outcome	Group 1	Group 2	Group 3	Group 4	p value	Effect size
O mass %	51.41±2.47	37.09±10.84	46.23±5.40	45.88±3.02	<0.001	ε^2=0.389
Ca mass %	32.95±2.61	39.53±1.95	36.58±5.00	40.80±2.82	<0.001	ε^2=0.431
P mass %	15.64±0.75	15.60±0.42	16.14±0.89	16.32±0.73	0.021	ε^2=0.121
F mass %	0.51±0.44	0.30±0.10	0.24±0.11	0.27±0.10	0.287	ε^2=0.014
Ca/P ratio	2.11±0.22	2.54±0.15	2.27±0.31	2.51±0.22	<0.001	ε^2=0.385

Oxygen

Mean O was highest in Group 1 (51.41±2.47%) and lowest in Group 2 (37.09±10.84%). The four-group difference was significant (H=24.805, df=3, $p<0.001$; ε^2=0.389). Group 1 showed significantly higher O than Groups 2, 3 and 4 after Bonferroni-adjusted Dunn testing. Comparisons among Groups 2, 3 and 4 were not statistically significant.

Calcium

Mean Ca increased from 32.95±2.61% in Group 1 to 40.80±2.82% in Group 4. The overall difference was significant (H=27.115, df=3, $p<0.001$; ε^2=0.431). Group 1 had significantly lower Ca than Group 2 ($p<0.001$) and Group 4 ($p<0.001$). Other adjusted pairwise comparisons did not reach significance.

Phosphorus and fluorine

P differed significantly overall (H=9.746, df=3, p=0.021; ε^2=0.121), although the numerical range was narrow. The only significant adjusted pairwise difference was between Group 2 and Group 4 (p=0.045), with higher P in Group 4. F was present only in trace quantities and did not differ significantly among groups (H=3.776, df=3, p=0.287; ε^2=0.014).

Calcium-to-phosphorus ratio

The Ca/P ratio was lowest in Group 1 (2.11±0.22), intermediate in Group 3 (2.27±0.31), and highest in Groups 2 (2.54±0.15) and 4 (2.51±0.22). The overall difference was significant (H=24.557, df=3, $p<0.001$; ε^2=0.385). The elemental pattern therefore differed according to both chelator and laser activation.

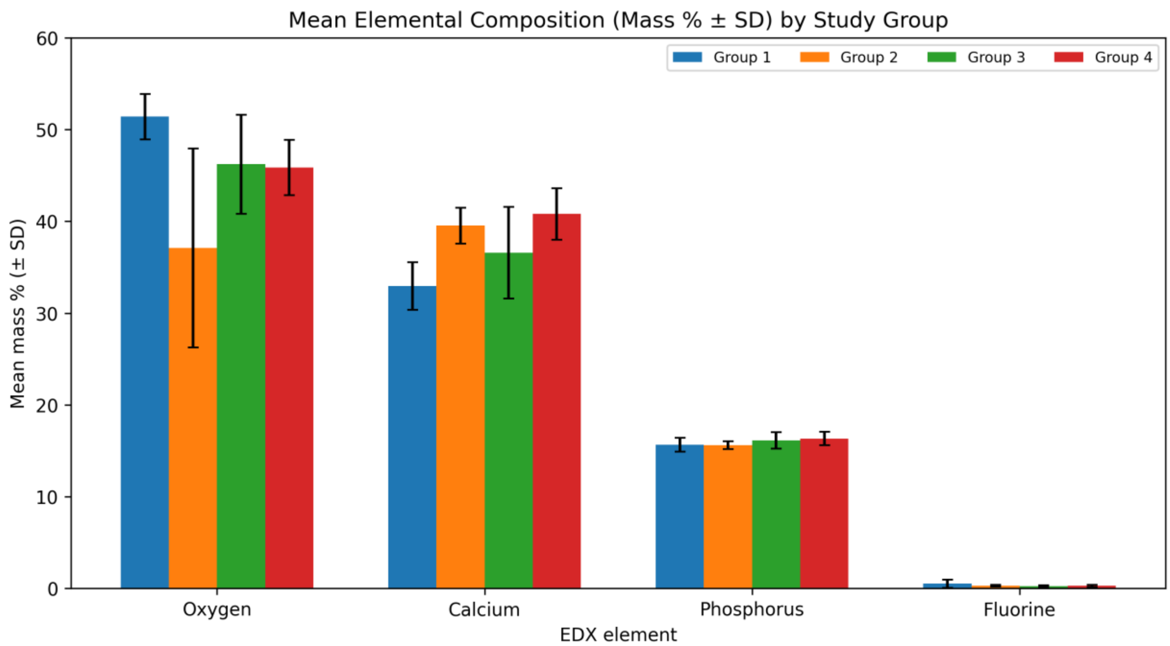

Fig. 1: Grouped bar graph of mean elemental composition (Mass % ± SD) across the four study groups.

The bar graph demonstrates that Group 4 (HEDP with diode-laser activation) showed the highest mean surface calcium (40.80 ± 2.82%) and phosphorus (16.32 ± 0.73%) values, whereas Group 1 (17% EDTA without laser activation) showed the lowest mean calcium value (32.95 ± 2.61%). Laser activation produced a marked change in the elemental profile, particularly for EDTA: Group 2 showed lower oxygen (37.09 ± 10.84%) and higher calcium (39.53 ± 1.95%) than Group 1, supporting greater exposure of the underlying mineralized dentin surface after activation.

IV. DISCUSSION

The present study demonstrated that the irrigation protocol influenced the elemental composition detected on apical root dentin. The null hypothesis was rejected for O, Ca, P and Ca/P ratio, but retained for F. The largest treatment-associated effect was observed for Ca, followed by O and Ca/P ratio. These findings support the concept that chelation and activation alter the chemical characteristics of the dentin surface, although EDX surface mass percentages should not be equated directly with whole-dentin mineral loss.

EDTA is effective for smear-layer removal because it chelates calcium from the inorganic dentin phase. Earlier chemical and EDX investigations have shown that EDTA can reduce mineral content and alter the Ca/P relationship [2–4,16,17]. In the present data, EDTA without laser showed the lowest mean Ca and Ca/P ratio, consistent with a more demineralized surface profile. Nogueira et al. similarly reported significant mineral loss with NaOCl/EDTA protocols, whereas HEDP-containing protocols retained higher Ca and P values [2].

HEDP differs from EDTA because it is a weaker chelator that is compatible with NaOCl for a clinically useful period, allowing chelation to occur throughout instrumentation. Rath et al. described less aggressive ultrastructural and compositional changes with continuous NaOCl/HEDP than with sequential NaOCl/EDTA [5]. Kfir et al. found comparable cleanliness with reduced erosion for an HEDP-based approach [7], and high-resolution micro-CT work demonstrated shallower erosion with HEDP than EDTA [8]. The ex-vivo study by Ballal et al. also suggested that continuous HEDP chelation was less detrimental to mechanical properties than sequential EDTA chelation [9].

Continuous chelation may additionally reduce accumulation of inorganic debris during preparation. Morago et al. reported a high proportion of smear-free tubules with NaOCl/HEDP while maintaining antimicrobial activity [18], and another study found that the antimicrobial activity of NaOCl/HEDP was less affected by the presence of smear layer than NaOCl alone [17]. Giardino et al. further demonstrated that addition of HEDP can modify the physicochemical behavior of NaOCl while maintaining useful dentin disinfection [18]. These findings provide a mechanistic basis for the clinical interest in HEDP-based continuous irrigation.

Activation can alter the effect of the irrigant by increasing fluid movement and contact with canal walls. Aoun et al. found that high-power sonic activation improved debris removal with Dual Rinse HEDP and enhanced smear-layer removal particularly in the apical third [6]. For diode lasers, Koçak et al. observed reduced smear-layer scores when laser irradiation was combined with EDTA or QMiX, although complete apical cleaning was not achieved [13]. Alfredo et al. showed that 980-nm diode irradiation can produce solution-dependent morphological changes in radicular dentin [14]. Amin et al. likewise reported that combining EDTA with diode-laser application improved smear-layer removal compared with several nonactivated protocols [12].

The present Group 4 (HEDP plus laser) had the highest mean Ca and P values, while both laser-activated groups had higher Ca/P ratios than their corresponding nonactivated groups. This may reflect greater removal of surface deposits and exposure of mineral-rich dentin rather than mineral gain. The distinction is important: EDX quantifies the relative elemental composition of the irradiated surface and cannot independently determine the depth or total amount of demineralization. Morphologic assessment and complementary techniques such as micro-CT, microhardness testing or chemical analysis are needed to characterize erosion comprehensively.

The relevance of the laser–HEDP combination is supported by the 2025 SEM study of Kottur et al., in which 980-nm diode laser combined with Twin Kleen improved smear-layer removal, particularly apically [15]. However, the current study differs by focusing on elemental surface composition rather than smear scores alone. Recent systematic reviews conclude that HEDP used in continuous chelation is generally comparable with, and in several studies superior to, conventional sequential irrigation for smear-layer removal, while also noting substantial heterogeneity in concentrations, activation methods and evaluation systems [10,11].

From a clinical perspective, an irrigation protocol should achieve chemical cleaning without unnecessarily compromising dentin. The present findings suggest that HEDP, especially when combined with diode-laser activation, produces a distinct mineral-exposure pattern and warrants further investigation. They do not establish superiority of one protocol for clinical outcomes, because this was an in-vitro surface-analysis study and did not evaluate long-term fracture resistance, sealer penetration, bacterial survival or treatment success.

Limitations

The study was performed on extracted single-rooted premolars under standardized laboratory conditions. EDX provides relative elemental mass percentages from the analyzed surface and does not quantify the three-dimensional depth of dentin erosion. Laser parameters, fiber movement, canal anatomy, irrigant volume and exposure time may influence the findings. Future studies should combine SEM–EDX with micro-CT, microhardness, fracture resistance, sealer interaction and microbiological outcomes.

V. CONCLUSION

Within the limitations of this in-vitro study, 17% EDTA and HEDP produced different elemental surface profiles in apical root dentin, and diode-laser activation further modified these profiles. Significant differences were observed for O, Ca, P and Ca/P ratio, whereas F remained unchanged. HEDP with laser activation showed the highest mean surface Ca and P values, while laser-activated EDTA and HEDP produced the highest Ca/P ratios. These findings indicate altered surface mineral exposure after chelation and laser activation; they should not be interpreted as direct proof of bulk mineral gain or loss.

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