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Original research article

Comparison of the smear layer- and debris-removal abilities and the effects on dentinal microhardness of 5% and 17% EDTA solutions used as final irrigants: *in vitro* study

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Abstract

Objective: The aim of this *in vitro* study was to evaluate the effects on dentinal microhardness, and the smear layer- and debris-removal properties, of 5% and 17% ethylenediaminetetraacetic acid (EDTA) solutions when used as final irrigants.

Materials and Method: Seventy extracted single-root human mandibular premolars were endodontically instrumented and distributed into 4 groups, according to the final irrigant: G1: 17% EDTA + 2.6% NaOCl (applied for 3 min and 1 min, respectively), G2: 5% EDTA + 2.6% NaOCl, G3 (control): saline + 2.6% NaOCl, and G4 (control): saline. Teeth from G1–G3 were split longitudinally, and scanning electron micrographs were obtained at 2 and 6 mm from the apex for smear layer and debris analyses. Teeth in all groups were sectioned horizontally, and Vickers microhardness values were measured at 500, 1000, and 1500 µm from the canal lumen. Data were statistically analyzed at the $p<0.05$ level.

Results: Smear layer scores were significantly greater at the 2-mm vs the 6-mm level in both EDTA groups, with no significant difference between EDTA groups at either level. Significantly less smear layer was found in the 17% EDTA group compared to the control at the 2-mm level. A statistically significant difference in microhardness among groups was found only at the 1500-µm level, with the 17% EDTA group exhibiting the lowest microhardness values.

Conclusion: The 5% and 17% EDTA solutions were equally effective at removing the smear layer and debris from instrumented root canal surfaces. However, the 5% EDTA solution did not decrease the microhardness of dentin like 17% EDTA.

Keywords: Anisotropy; chelating agents; endodontics; root canal irrigants; root canal therapy

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Introduction

Irrigating solutions are used during endodontic treatment to dissolve organic or necrotic tissues, disinfect the root canal system, facilitate instrumentation through lubrication, prevent packing of dentinal chips in the apical region, and remove debris and the smear layer.[1] Current endodontic instrumentation techniques inevitably produce a smear layer that covers the root canal walls and the openings of dentinal tubules. This layer contains organic and inorganic substances, including fragments of odontoblastic process, microorganisms, and necrotic materials.[2,3] In addition to the smear layer, another concern for endodontists is the debris of dentinal shavings, residual pulp tissue, as well as microorganisms and their byproducts that accumulate on instrumented root canal walls. For predictable treatment outcomes, this debris must be removed from the root canal system. Debris removal is mainly performed through irrigation.

Solutions, such as citric acid, maleic acid, and ethylenediaminetetraacetic acid (EDTA), are capable of

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removing the smear layer.[4,5] Among these solutions, 17% EDTA is a popular chelating agent that reacts with calcium ions in dentin and forms soluble calcium chelates. The chelation reaction theoretically alters the calcium-phosphorus ratio of hydroxylapatite and the microstructure. The use of 17% EDTA has been associated with reduced microhardness values for the root canal dentin,[4-6] which may have adverse effects on the sealing ability and adhesion of dental materials.[7]

The ability of 17% EDTA to remove the smear layer is well documented,[3] with some studies suggesting similar abilities for lower concentrations of EDTA.[8-10] In a previous study, similarly, we found that a 5% EDTA solution effectively removed the smear layer, and this allowed greater depth of tubule penetration for a mineral trioxide aggregate-based, salicylate resin root canal sealer.[11] However, little is known about the effects of lower EDTA concentrations on the dentinal microhardness.[12] Therefore, the aim of this study was to evaluate the dentinal microhardness effects and smear layer- and debris-removal abilities of 5% and 17% EDTA solutions when used as final irrigants in instrumented root canals.

Materials and Method

This study was approved by the Ethical Review Board of the Faculty of Dentistry of Ankara University (36290600/82).

Selection and preparation of teeth

Seventy extracted single-root and single-canal, noncarious human mandibular premolars with straight roots were selected for the study. Lengths of the teeth were between 20 and 22 mm. A standard access cavity was prepared, and the pulp was removed. The working length was established 1 mm short of the apical foramen. The root canal was instrumented by using ProTaper Universal rotary NiTi files to size F3, according to the manufacturer's instructions (Dentsply Maillefer, Ballaigues, Switzerland). Between each file, the root canal was irrigated with 2 mL of 2.6% NaOCl by using a 27-gauge needle. The active chlorine concentration of the NaOCL solution was confirmed with a colorimetric test kit prior to use (Sutest, Damla Kimya, Ankara, Turkey). The total instrumentation time for each tooth ranged from 5 to 7 minutes.

Experimental and control groups

Teeth were distributed into 2 experimental and 2 control groups, according to the final irrigant. Application times of the irrigants were according to a previous study.[11] In Group 1 (17% EDTA + 2.6% NaOCl; n = 20), the root canal was irrigated with 5 mL of a 17% EDTA solution (Fluka Biochemika, Steinheim, Switzerland, pH 7.3) for 3 minutes, followed by irrigation with 5 mL of a 2.6% NaOCl solution (1 minute) and 2 mL of distilled water. In Group 2 (5% EDTA + 2.6% NaOCl, n = 20), irrigation was performed identically to Group 1, except that a 5% EDTA solution (pH 7.3) was used instead of the 17% solution. In Group 3 (first control; Saline + 2.6% NaOCl, n = 20), irrigation was performed identically to Group 1, except that a saline solution (0.9% NaCl) was used instead of EDTA.

A second control group (Group 4) was used for the microhardness test. In Group 4 (Saline, n = 10), 10 mL of saline solution were used for irrigation instead of EDTA and NaOCl, followed by irrigation with 2 mL of distilled water. Unlike the other groups, root canals in Group 4 were irrigated with saline solution during instrumentation.

Evaluation of the smear layer and debris

Ten teeth each from Groups 1 to 3 were selected randomly and split longitudinally into mesial and distal halves. Half of these teeth were dehydrated in a graded ethanol series (30%, 40%, 50%, 60%, 70%, 80%, 90%, and 96%; 30 minutes each) and kept in an incubator at 37 °C for 1 day. Samples were mounted on aluminum stubs and sputter-coated with gold-palladium. An operator (AG) who was experienced in the analysis of tooth specimens and blinded to the aim of the study obtained scanning electron micrographs (JSM-6060 LV, JEOL, Tokyo, Japan) of the teeth at ×1000 magnification.[13] Three consecutive micrographs were obtained along the midline of the canal, at 2 and 6 mm from the apex. Groups were masked by assigning a randomly generated 3-digit numeric code to each tooth. Two observers (GKD and GK) evaluated the smear layer and debris, using previously described 3-step scoring systems,[13] as shown in Figure 1.

Examiner calibration

Both observers scored 10% of the photographs together and then scored the remaining 90% of the photographs independently. After 6 weeks, each observer performed a second reading on a randomly selected 50% of the photographs. Inter- and intra-observer agreement levels were calculated by kappa statistics (unweighted Cohen's κ). Photographs used for calibration were not included in the κ analyses. Photographs given different scores by the two observers were discussed until a consensus agreement was achieved. After consensus agreement, the photographs were unmasked.

Microhardness evaluation

Microhardness was evaluated similarly to a previous study.[14] Briefly, the remaining 10 teeth in each group were sectioned horizontally along the middle of the tooth length by using a rotating diamond saw under water cooling. Coronal fragments were embedded in acrylic resin blocks, with the surface exposed. The surface was ground under running water by P 1000 silicon car-

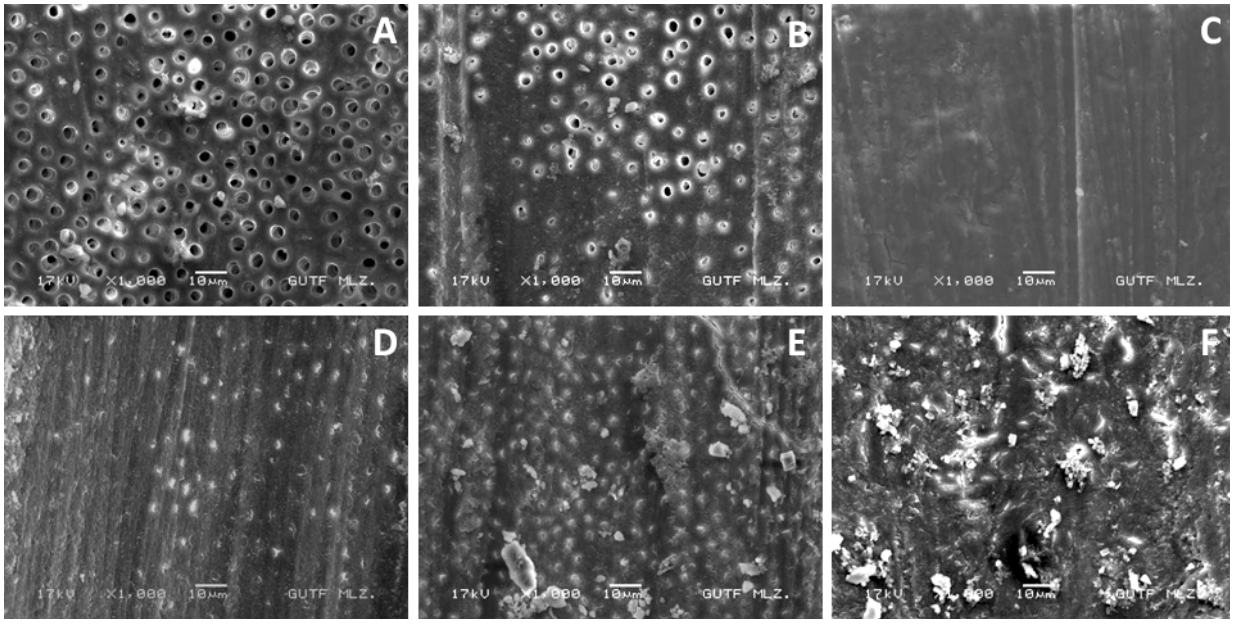

Figure 1. *Representative scanning electron micrographs for each smear layer (A-C) and debris score (D-F) (×1000 magnification; bar represents 10 µm); (A) Score 0: All dentinal tubules are open, and no smear layer is present. (B) Score 1: Some dentinal tubules are open, with smear layer covering some openings of the dentinal tubules. (C) Score 2: All dentinal tubules are covered by smear layer. (D) Score 0: No debris is present. (E) Score 1: A few debris particles are present. (F) Score 2: A large amount of debris is present.*

bide abrasive paper (Presi, Grenoble, France), followed by polishing with paste (6-µm grain size; Preparations Diamantees Mecaprex, Presi) in a polishing machine (Mecapol P230, Presi).

Groups were masked by assigning a randomly generated 3-digit numeric code to each sample. One observer (GKD) blindly analyzed all samples. A Vickers microhardness tester (HMV-700, Shimadzu Corp., Tokyo, Japan) was used at a load of 100 g for 10 seconds. Three indentations were made at 500, 1000, and 1500 µm from the canal lumen, with 100 µm distance between indentations. The indentations were observed under stereomicroscope at ×40 magnification. Vickers hardness values were provided by the built-in calculation program of the instrument. The arithmetic mean was calculated for each distance. The procedure was done on both the buccal and lingual sides (Figure 2), and the values were pooled.

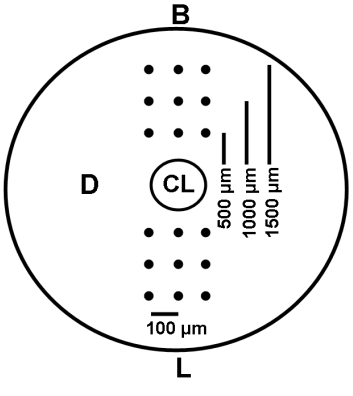

Figure 2. *Schematic illustration of the root section, as well as the indentation points and locations in the microhardness test (B: buccal, CL: canal lumen, D: dentin, L: lingual).*

Statistical analysis

Microhardness data were analyzed by the Kolmogorov–Smirnov test to determine the normality of the distribution, followed by analysis of variance (ANOVA) and the Tukey HSD test. For smear layer and debris analyses, differences within groups (between the 2- and 6-mm levels) were analyzed by the Wilcoxon signed-rank test; differences among groups (at the 2- and 6-mm levels) were analyzed by the Kruskal–Wallis test, followed by pairwise comparison by the adjusted Mann–Whitney U test. A p value less than 0.05 was considered statistically significant.

Results

Inter- and intra-observer agreement levels for smear layer and debris

For smear layer and debris, 180 photographs were assessed. Cohen's κ values for interobserver agreement for the smear layer and debris were 0.81 and 0.62, respectively (0.61–0.80: substantial agreement, and 0.81–1: almost perfect agreement).[15] For intraobserver agreement, κ values for the smear layer and debris were 0.88 and 0.69, respectively, for GDK and 0.84 and 0.69, respectively, for GK. Consensus agreement was sought in 18 photographs for the smear layer and 33 photographs for debris. The difference in these photographs was always at a magnitude of 1 score.

Smear layer

Smear layer scores (Table 1) were greater at the 2-mm level compared to the 6-mm level for 17% EDTA (p=0.034) and 5% EDTA (p=0.011). The first control

group had a smear layer score of 2 in all photographs. Differences were found between the groups at the 2- and 6-mm levels (p=0.008 and 0.000, respectively; Kruskal–Wallis test). At the 2-mm level, less smear layer was found in the 17% EDTA group compared to the first control group (p=0.007), with no other significant differences. At the 6-mm level, less smear layer was found in both EDTA groups compared to the first control group (p=0.000 for both), with no significant difference between the EDTA groups.

Debris

Debris scores were similar at the 2- and 6-mm levels for the 5% EDTA, 17% EDTA, and first control groups (p>0.05). No significant difference was found between the groups at the 2- or 6-mm level (p>0.05, Table 1).

Microhardness

Vickers microhardness values gradually increased from the superficial to the deep-dentin layer for all groups (no statistical analysis done). A statistically significant difference among groups was only found at 1500 µm from the canal lumen (p = 0.011; ANOVA; Figure 3). The 17% EDTA group had a lower microhardness value than the 5% EDTA (p=0.024), first control (p=0.037), and second control groups (p=0.034). No significant difference was found between other groups (p>0.05).

Discussion

The 5% and 17% EDTA solutions exhibited similar smear layer-removal abilities. Dentinal microhardness at the deep-dentin layer was reduced with the use of 17% EDTA but not with 5% EDTA. Debris removal was similar in all groups.

Final irrigation regimes (including application time of the irrigants, irrigation sequence, etc.) for smear layer removal in instrumented root canals differ greatly among studies.[4,5,16,17] In one study, it was shown that application of 15% EDTA and 1% NaOCl for only 1 minute yielded similar results to application of the same solutions for 3 or 5 minutes.[18] However, in the present study, the application time and the irrigation sequence were chosen with reference to our primary data;[11] thus we adopted an application time of 3 min for EDTA, and 1 min for NaOCl.

Table 1. *Smear layer and debris scores in Groups 1–3 [median; mean (standard deviation), n=30] and statistical comparisons.*

Smear layer	2 mm	6 mm
Group 1 (17% EDTA + 2.6% NaOCl)	1; 1.27 (0.64) Aa	1; 0.57 (0.45) Ab
Group 2 (5% EDTA + 2.6% NaOCl)	2; 1.50 (0.71) ABa	1; 0.63 (0.48) Ab
Group 3 (Saline + 2.6% NaOCl)	2; 2.00 (0.00) Ba	2; 2.00 (0.00) Ba
Debris		
Group 1 (17% EDTA + 2.6% NaOCl)	1; 0.70 (0.64) Aa	0; 0.20 (0.32) Aa
Group 2 (5% EDTA + 2.6% NaOCl)	1; 0.57 (0.52) Aa	0; 0.40 (0.41) Aa
Group 3 (Saline + 2.6% NaOCl)	1; 0.70 (0.62) Aa	0; 0.23 (0.35) Aa

Within each smear layer and debris comparison, different capital letters (for columns) and different lowercase letters (for rows) indicate a statistically significant difference (p<0.05).

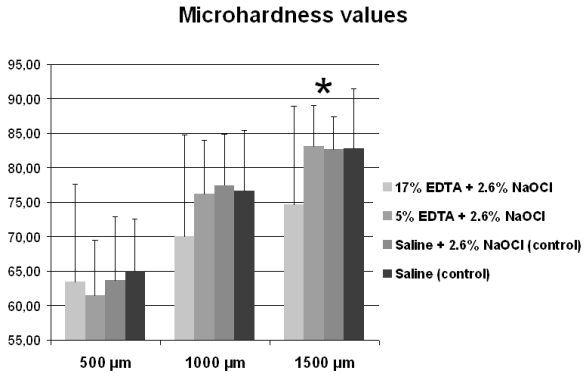

Figure 3. *Bar graph (means and standard deviations) showing the microhardness values for experimental groups and controls at each distance (n = 20). Asterisk (*) indicates the distance where a statistical significance was found among groups (p<0.05).*

The observed similar effectiveness of low and conventional concentrations of EDTA in removing the smear layer is consistent with findings from previous studies.[8-11] Using a continuous dual irrigation regime of even a weaker concentration of EDTA (1%) in combination with different concentrations of NaOCl, Kaya *et al.*[19] revealed smear-free dentinal surfaces in most teeth instrumented by Self-Adjusting File. Thus, the current study supports the conclusion of previous literature that solutions with low EDTA concentrations can be as effective in removing the smear layer as the conventional 17% EDTA solution.

Debris removal was similar in all groups regardless of the final irrigant. This finding supports the results of a previous study, in which there was no significant difference among different irrigants delivered through a conventional syringe or applied by ultrasound.[20] The finding that no significant difference existed for debris between the 2 levels is in line with the findings of some previous studies.[16,21] However, Klyn *et al.*[22] found a greater amount of debris at the 1-mm level compared to the 3- or 5-mm level. Root canals in the current and supporting studies were irrigated with larger volumes of solution compared to root canals in Klyn *et al.*[22] which may explain the discrepancy in results.

Root sections exhibited a gradually increasing microhardness profile from the superficial to deep-dentin layer. This finding can be explained by the increased tubular diameter and density, and reduced amount of intertubular dentin, as the pulp chamber is approached.[23] Dentinal microhardness was significantly reduced only at the deep-dentin layer (1500 µm) when 17% EDTA was used. This result was probably due to an agent-substrate relationship, as the EDTA found a greater proportion of inorganic matter (calcium ions) to react with in the deeper parts of the dentin. This assumption is supported by previous reports of a lower mineral/collagen ratio and higher collagen content of intercuspal superficial dentin compared to deep dentin.[24] Similarly,

Ghisi *et al.*[25] found significantly lower microhardness values for the deep-dentin layer when using 17% EDTA compared to superoxidized water, whereas the microhardness values were comparable at a point close to the lumen.[25]

Contrary to our findings, others have found that EDTA reduced the microhardness even at the level of superficial dentin (within 500 µm).[4,6,12,17,26,27] This discrepancy may relate to the anisotropy (directional dependency) of dentin, due to its tubular organization. The current and supporting studies[25] used horizontal sections of roots. In contrast, the contradicting studies used longitudinal sections, such that the samples were approached for measurement from different axes. Another explanation may be the variations between the irrigation protocols followed in these studies.

Another new finding of the current study was that the use of 5% EDTA did not significantly affect the dentinal microhardness. This finding differs from that of Akcay and Sen,[12] who found that a 5% EDTA solution significantly reduced dentinal microhardness at the level of the superficial dentin. The reason for this discrepancy may be due to the methodological difference discussed above.

Avoiding the microhardness reduction at deep dentin through use of a low-concentration EDTA may help preserve the original mineral integrity and the resistance of the tooth against fracture or caries. Another clinical advantage of using a low-concentration EDTA was suggested in a previous study.[28] In this study, the contact angle between the endodontic sealer and dentin decreased in the 3% EDTA group but increased in the 15% EDTA group. Theoretically, this would affect the sealer adaptation and penetration into the dentinal tubules. Furthermore, considering the high cost of EDTA, use of a low concentration may be advantageous.[1]

Conclusion

In conclusion, when used in combination with NaOCl, 5% EDTA solution as the final irrigant removed the smear layer and debris from instrumented root canal surfaces as effectively as 17% EDTA solution, and did not decrease the microhardness of dentin like 17% EDTA. These findings suggest that 5% EDTA solution can function successfully as a final irrigant.

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Conflict of interest disclosure: The authors declare no conflict of interest related to this study.

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Son yıkamada kullanılan %5 ve %17 EDTA çözeltilerinin smear tabaka ve debriz uzaklaştırma etkinliklerinin ve dentin mikrosertliğine etkilerinin karşılaştırılması: *in vitro* çalışma

Özet

Amaç: Bu *in vitro* çalışmanın amacı, %5 ve %17 etilendiamintetraasetik asit (EDTA) çözeltilerinin son yıkama çözeltisi olarak kullanıldıklarında, smear tabaka ve debris uzaklaştırma etkinliklerinin ve dentin mikrosertliği üzerine etkilerinin karşılaştırılmasıydı.

Gereç ve Yöntem: Yetmiş adet çekilmiş, tek köklü, insan alt çene, küçük azı dişi endodontik olarak enstrümante edildi ve son yıkamaya göre 4 gruba dağıtıldı: G1: 17% EDTA + 2.6% NaOCl (sırasıyla, 3 dak. ve 1 dak. uygulandı), G2: 5% EDTA + 2.6% NaOCl, G3 (kontrol): salin + 2.6% NaOCl, and G4 (kontrol): salin. G1-G3 gruplarına ait dişler boylamasına ikiye ayrıldı ve smear ve debriz incelemesi için, apeksten 2 ve 6 mm mesafelerde tarama elektron mikrografları elde edildi. Ayrıca, tüm gruplardaki dişler horizontal olarak kesilerek, kanal boşluğundan 500, 1000 ve 1500 µm mesafelerde Vickers mikrosertlik ölçümleri yapıldı. Veri $p<0.05$ seviyesinde istatistiksel olarak incelendi.

Bulgular: Smear tabakası için ölçüm değerleri, her iki EDTA grubunda da 2 mm seviyesinde, 6 mm seviyesine göre anlamlı olarak daha yüksekti; EDTA grupları arasında herbir seviye için anlamlı fark yoktu. Kontrole göre, 2 mm seviyesinde, %17 EDTA grubunda anlamlı olarak daha az smear tabakası vardı. Dentin mikrosertliği bakımından, gruplar arasında istatistiksel olarak farklılık yalnızca 1500 µm mesafede görüldü; burada %17 EDTA grubu en düşük mikrosertlik değerlerini sergiledi.

Sonuç: Enstrümante kök kanalı yüzeyinden smear tabakası ve debris uzaklaştırma bakımından %5 ve %17 EDTA çözeltileri benzer etkinlik gösterdi. Ancak, %5 EDTA çözeltisi dentin mikrosertliğini %17 EDTA çözeltisi gibi azaltmadı.

Anahtar Kelimeler: Anizotropi; kelat edici ajanlar; endodonti; kök kanalı sulayıcıları; kök kanal tedavisi