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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF DIFFERENT ROOT CANAL IRRIGANTS ON THE MECHANICAL PROPERTIES OF ROOT DENTIN - AN IN VITRO STUDY

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ABSTRACT

Different concentrations of Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and Cetrimide have varied effects on the mechanical properties of endodontically treated teeth. **AIM:** This study aims to evaluate the mechanical properties of root dentin after exposure to different endodontic irrigating solutions. **Methods:** This in-vitro study evaluated the effect of different irrigation regimens on the flexural strength and Knoop microhardness of root dentin. The sample size was estimated to be a total of 56 samples (40 samples for flexural strength and 16 samples for microhardness). For flexural strength, dentin beams (12×2×1mm) were obtained from roots and randomly divided into five groups based on the irrigation regimen: Group I: 5% NaOCl + 17% EDTA, Group II: 5% NaOCl + 17% EDTA + 0.2% Cetrimide, Group III: 3% NaOCl + 17% EDTA. Group IV: 3% NaOCl + 17% EDTA + 0.2% Cetrimide, Group V (Control): Normal saline. Flexural strength was measured using a three-point bending test on a universal testing machine. while root dentin discs were tested for Knoop microhardness before and after irrigation. Statistical analysis was performed using SPSS (Version 26.0). Data were analyzed with descriptive statistics, paired t-test, and One-Way ANOVA followed by Tukey's HSD post hoc test, after confirming normal distribution. A p-value < 0.05 was considered statistically significant. **Results:** Flexural strength testing showed that Group 2 had the greatest reduction in strength, which was significantly lower than the control ($p < 0.05$), though not significantly different from other experimental groups. Groups 3 and 4 showed moderate, non-significant reductions. For microhardness, all experimental groups showed a decrease in Knoop values post-irrigation, but no statistically significant differences were found among them. **Conclusion:** High-concentration NaOCl with cetrimide significantly reduced dentin flexural strength, while microhardness changes were minimal; lower NaOCl levels caused less structural weakening, indicating a safer irrigation approach. Cetrimide's role as a surfactant improves penetration of irrigants but may amplify damage when used with strong oxidizers.

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INTRODUCTION

The main cause of pulpal infection is the bacterial invasion of the root canal system. The recommended solution is root canal treatment, which aims to eradicate bacteria and thoroughly disinfect the canals to maintain the tooth's proper function. Successful endodontic treatment depends on the thorough debridement of the root canal system through a combination of mechanical instrumentation and chemical disinfection, followed by effective sealing with appropriate materials (1)(2). Sodium hypochlorite (NaOCl) is the most commonly used irrigant in endodontics. Since NaOCl cannot remove the inorganic components of the smear layer, it is typically followed by a chelating agent like EDTA at a concentration of 15–17% for 1–2 minutes (3). There is no consensus on the optimal concentration of NaOCl for root canal irrigation. However, there is a general trend toward using higher concentrations due to their superior efficacy in disinfection and soft tissue dissolution. However, multiple researchers have stated that higher concentrations of NaOCl may irritate periapical tissues and compromise the mineralized structure of the root canal system (2). Additionally, Xu et al have reported that the degradation of root dentin caused by high-concentration NaOCl may increase the risk of fracture under functional loading (2). The effectiveness of irrigation relies not only on the irrigants's mechanism of action but also on their ability to contact microorganisms and tissue debris within the root canal systems. NaOCl and EDTA with a higher surface tension restrict the solution's ability to enter narrow spaces of the root canal system (4). Therefore, the efficacy of irrigating solutions can be enhanced by incorporating surface-active agents (5). Cetrimide (CTR) is a cationic surfactant, appearing as a white odorless powder, that not only reduces surface tension of fluids but also disrupts the cohesion between extracellular matrix polymers and bacterial cell walls, providing antibacterial effects (6,7). At a 0.2% concentration, CTR has been demonstrated to eliminate E. faecalis biofilm (7). Root canal irrigants, as previously discussed, can alter the mechanical properties of dentin, including its flexural strength. Since dentin comprises a significant portion of the tooth structure, any changes to its mechanical integrity following irrigation are likely to affect the overall strength of the tooth, which ultimately reduces the fracture resistance of endodontically treated tooth. Hence, the aim of this in vitro study is to evaluate the effect of different root canal irrigants on the mechanical properties of root dentin in extracted human teeth by evaluating the root dentin microhardness through Knoop's microhardness test and flexural strength through 3-point flexural strength test. The null hypothesis states that there would not be any significant difference in the flexural strength and microhardness of root dentin after exposure to different root canal irrigants.

MATERIALS AND METHODS

Preparation of the stock solution: To prepare a solution of 0.2% Cetrimide, 200mg of 100% cetrimide powder was diluted to 100ml of 17% EDTA solution. A magnetic stirrer was employed to combine the contents of cetrimide powder in the EDTA solution. The freshly prepared solution was stored in airtight amber bottles until use. (8,9)

Sample size calculation and selection: A total of 24 human-extracted maxillary canines with a single and straight root canal and fully formed roots were selected, and their selection was confirmed with digital radiographs. Teeth with calcifications, cracks, curvatures, or previous root canal treatment were excluded from the final sample. Of these, 20 teeth were used for the flexural test and 4 for the microhardness test. The teeth were cleaned and rinsed with distilled water and stored in 0.05% thymol to prevent bacterial growth. The sample size was estimated with G Power 3.1. software (Heinrich-Heine Universität), for both flexural and microhardness test based on the study by Xu H et al (4) with a statistical power of 80%, an alpha level of 5%, and with an effect size of 0.62, each group should contain 8 specimens per group for the flexural strength test. To observe a significant difference, for the Knoop microhardness test with a calculated effect size of 2.92, giving a sample size of 4 specimens per group for the microhardness test.

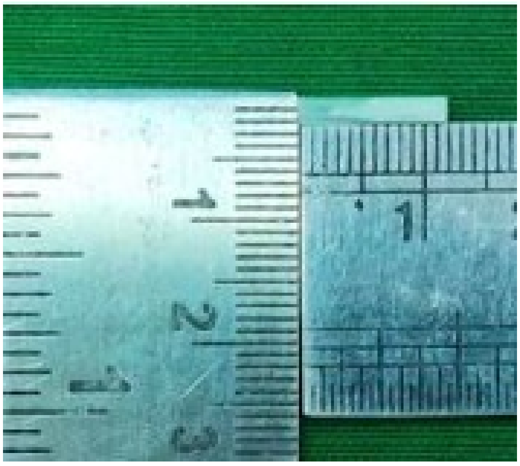

Figure 1. Dentin beam with dimensions 12x2x1mm

Specimen Preparation: Root Dentin Beams: (Based on study by Savaris et al) (10) 20 maxillary canines that met the inclusion criteria were selected and decoronated. The root segments were embedded in auto-polymerizing resin and were then sectioned longitudinally into 2 parts with a diamond disc under water coolant. Two root dentin beams measuring 12x2x1 mm were obtained from each tooth specimen, using a cutting machine (Isomet 1000; Buehler, Illinois, USA), with a total of 40 root dentin beams, and were randomly divided among the five groups. (Fig. 1)

Root Dentin Discs

4 additional maxillary canines that met the inclusion criteria were selected and decoronated. Root canals were prepared using the crown-down technique with Protaper Gold rotary files (X-Smart endomotor) up to size F4, following preset speed and torque settings. Normal saline was used for irrigation after each file, and files were discarded after five canals. From each of the four roots, four 2 mm discs were obtained and evenly distributed into groups I–IV to ensure equal representation of root regions (Fig. 2). Each disc was halved; one half served as the control by immersion in saline for baseline KHN measurement. The remaining tooth surface was then coated with nail varnish, leaving only the canal wall exposed.

Figure 2. 2mm dentin disc

Grouping: A total of 40 root dentin beams measuring 12x2x1 mm and 16 dentin discs with 2mm thickness were obtained from freshly extracted maxillary canines. The dentin beams were divided into five groups: Group I-V (n=8) and disc were divided into four groups: group I-IV (n=4), and were exposed to irrigating solutions.

Group 1:5%NaOCl for 5 mins and 17%EDTA for 2 mins,

Group 2: 5% NaOCl for 5 mins and 17% EDTA with 0.2% Cetrimide for 2 mins,

Group 3:3% NaOCl for 5 mins and17% EDTA for 2 mins,

Group 4:3%NaOCl for 5 mins and 17%EDTA with 0.2% Cetrimide for 2 mins, Group 5: 0.9% Normal Saline for 5 mins

At the end of each irrigant immersion treatment period, the samples were rinsed with distilled water and dried.

Figure 3. Instron Universal Testing Machine

Figure 4. Dentine beams subjected to a three-point bending test

Flexural test: Each dentin beam was subjected to a three-point bending test with a universal testing machine (Instron 3000; USA) (Fig. 3). The specimens were positioned on two points to create a 10-mm test span with the cross-head (crosshead speed: 0.75 mm/min) centered in this span (Fig. 4). The maximum force required to fracture each specimen was noted. The flexural strength was calculated in MPa, according to the formula:

Flexural strength (MPa) = (3xPxL)/(2xbxd2)

Where P = load to fracture (N), L = support length (mm), b = beam width (mm), and d = beam thickness (mm).

Knoop Microhardness test: The Knoop Microhardness test was performed on the dentin discs before and after exposure to the irrigants. After following the irrigation protocol for all the four groups, the specimens were washed with saline to remove any remaining residual solution. Following this, the nail varnish was removed, followed by the flat surfaces of the discs were polished using a series of SiC papers (up to 1200 grit) and a diamond paste. The specimens were then cleaned ultrasonically and tested immediately. Knoop hardness (KHN) was measured on a micro-hardness testing machine with a Knoop diamond indenter. The long diagonal of the indenter was kept perpendicular to the radial direction of the disc. Three indentations were made on each specimen with a force of 50 g applied with a dwell time of 20 seconds, and the average of these three readings was recorded (Fig. 5).

Figure 5. Long diagonal of the indenter perpendicular to the radial direction of the disc

Statistical analysis: The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to verify the normal distribution of the data before statistical comparison. The results were then analysed using one-way ANOVA with the level of significance set at 0.05. Multiple comparisons among the different groups were performed using the Tukey HSD post hoc test. The paired t-test was conducted for the Knoop microhardness test, comparing pre- and post-microhardness values for the four groups.

RESULTS

The results of the study were evaluated and tabulated as follows.

Table 1. Descriptive statistics on the flexural strength among six groups

Groups (n=8)	Flexural Strength (MPa) Mean ± SD
GROUP I	161.48 ± 78.51
GROUP II	106.43 ± 64.31
GROUP III	176.81 ± 95.76
GROUP IV	174.32 ± 81.01
GROUP V	245.47 ± 96.79

Table 2. Paired Sample t-Test Statistics for microhardness

Groups (n =4)	Root Dentin Microhardness (Mean KHN ± SD)	
	Pre-Treatment	Post-Treatment
GROUP I	55.80 ± 23.07	34.75 ± 14.19
GROUP II	55.62 ± 10.09	29.78 ± 9.91
GROUP III	32.73 ± 4.08	40.10 ± 10.80
GROUP IV	30.60 ± 4.34	38.72 ± 5.16
GROUP V	42.1 ± 2.45	28.42 ± 3.61

Flexural Test: The mean flexural strength values varied from 106.43 MPa (Group II) to 245.47 MPa (Group V), indicating substantial differences among groups, with Group V showing the highest mean value and Group II showing the lowest mean value of flexural strength ($p= 0.018$). (Table 1).

The one-way ANOVA indicated significant differences in flexural strength among the five groups. To pinpoint which specific groups differed, Tukey's HSD post hoc test was performed and it was found that a statistically significant difference was observed between Group II and V ($p = 0.018$) for both maximum force and flexural strength. Group V demonstrated significantly higher maximum force and flexural strength compared to Group II. No other pairwise comparisons showed significant differences, indicating that the other groups performed similarly in terms of maximum force and flexural strength.

Knoop Microhardness Test: The mean value shows a consistent decline from pre to post-intervention across all groups. In SI the microhardness decreased from 55.80±23.07 to 34.75±13.07. In SII the microhardness decreased from 55.62±10.09 to 29.28±18.29. In SIII the microhardness decreased from 38.72±8.55to 30.60±6.23 and in SIV the microhardness decreased from 42.10±18.74 to 28.4250±4.50. There were no statistically significant differences seen in all four groups. (Table 2). The One-way ANOVA revealed no statistically significant differences in hardness among the groups when compared before and after treatment. The treatment appears to affect all groups similarly.

DISCUSSION

The present study evaluated the effect of three different root canal irrigants on the mechanical properties of root dentin. There was a significant difference in the flexural strength between the experimental and control groups. However, there was no significant difference in Knoop microhardness before and after exposure to different root canal irrigants. Therefore, the null hypothesis was partially rejected. Among the available options, NaOCL is universally recognized as the primary irrigant due to its proven tissue-dissolving ability and strong antimicrobial properties, even at concentrations as low as 0.5% (11). However, NaOCL alone is ineffective at removing the inorganic components of the smear layer (12). The American Association of Endodontists (AAE) recommends the use of a decalcifying agent like EDTA for at least two minutes to facilitate smear layer removal (13). Notably, NaOCL and EDTA, the two most widely used irrigants, are chemically incompatible (14). Therefore, they should not be mixed directly, and an intermediate rinse with saline or distilled water is recommended when used sequentially (13,14). Surfactants are wetting agents added to the irrigants to reduce the surface tension of the irrigating solutions. CTR is commonly added to EDTA to improve its clinical performance (2). Adding CTR to EDTA does impact mechanical properties, especially dentin microhardness, but the extent of impact depends on the concentration of cetrimide and the exposure time during irrigation (8). CTR, when added to EDTA, primarily acts as a surfactant, and it can slightly amplify the softening effect on dentin if used in higher concentrations (5). However, at lower concentrations (0.2%–0.25%), studies have shown that CTR addition does not significantly worsen EDTA's softening effect on dentin (8). NaOCl in higher concentrations causes dentin collagen structure degeneration, affecting dentinal microhardness and reducing fracture resistance of endodontically treated teeth (2). The deproteination by higher concentrations of NaOCl may cause a significant drop in dentin microhardness. When a chelator follows this, it further exacerbates the effect of NaOCl (15). In the present study, flexural strength was reduced in all the experimental groups. Group 2 showed a significant decrease in flexural strength amongst all the experimental groups compared to the control group (Group 5). This can be attributed to the fact that the combination of 5% NaOCl and 17% EDTA with 0.2% CTR exerted a synergistically destructive effect on dentin, causing significantly greater flexural strength reduction compared to saline (16). This effect arises from the intensified demineralization, collagen degradation, and surface destabilization mediated by the chemical interactions of these agents, particularly at higher NaOCl concentration (17). CTR enhances tissue penetration and lowers surface tension, thereby increasing the depth and rate of demineralization and collagen matrix damage (16).

Microhardness testing is considered the gold standard for observation of hard tissue changes to different root canal irrigants and is a strong predictor for Young's Modulus and Yield stress (18). All groups in the present study showed a decrease in dentin microhardness, although the reduction was not statistically significant. This suggests that exposure to NaOCL, either alone or in combination with chelators, decreases dentin microhardness. These findings are consistent with a study by Zhang et al (19) and Akcay and Sen et al (8). Zhang et al. evaluated the effect of NaOCl/EDTA with the addition of cetrimide on dentin microhardness. They observed a reduction in microhardness following irrigation; however, adding 0.2% cetrimide did not cause a statistically significant further decrease compared to NaOCl/EDTA alone (19). According to the study done by Akcay and Sen et al, they evaluated the effect of adding surfactant to EDTA on the microhardness of root dentin. They found that 0.2% CTR + EDTA solution caused a slight decrease in microhardness, although this reduction was not statistically significant (8). As an experimental study, extracted teeth might not fully represent the anatomical and structural variations found in different age group patients. The study focused mainly on the mechanical parameters like flexural strength and microhardness without SEM imaging or spectroscopy to evaluate surface erosion, collagen degradation or mineral loss. This study tested fixed concentrations of cetrimide and NaOCl. Evaluation of different concentrations could have revealed dose-dependent effects on root dentin. Although the study found statistically significant flexural strength reduction in group 2 compared to the control group, the absence of significance in other intergroup comparisons may reflect a limited sample size or statistical power.

CONCLUSION

Within the limitations of this experimental study, it can be concluded that significant flexural strength reduction occurred in the group treated with 5% NaOCl and 17% EDTA with 0.2% Cetrimide as compared to normal saline. Microhardness reduction was observed in all experimental groups subjected to different irrigants, though not significantly. Future laboratory studies and clinical trials should incorporate a wider variety of tooth types, a larger sample size, and different irrigant concentrations to more accurately simulate clinical conditions. Similarly, studies are needed to check the antibiofilm effectiveness, cytotoxicity of the novel endodontic irrigant.

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