The effect of final irrigation solution and irrigation techniques on the flexural strength and elastic modulus of root canal dentin: an experimental study

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ABSTRACT

***Introduction**: Root canal irrigation facilitates debris removal, lubrication, and the dissolution of organic and inorganic tissues while preventing smear layer formation. However, the chelating properties of final irrigants may deplete calcium ions from the dentin, potentially compromising the flexural strength and modulus of elasticity of the root canal walls. This study aims to analyze the effects of various final irrigants and agitation techniques on these mechanical properties of radicular dentin. **Methods**: Forty-five mandibular premolars were prepared and randomly divided into three groups (n=15). Group 1 was irrigated with 17% EDTA, Group 2 with 0.2% chitosan nanoparticles, and Group 3 with Ultra Clean. Each group was further subdivided into three subgroups (n=5) based on the agitation technique used: manual (A), sonic (B), and ultrasonic (C). Flexural strength and elastic modulus were measured using a Universal Testing Machine (UTM). **Results:** Two-way ANOVA showed that the type of irrigating solution, agitation technique, and their interaction significantly affected flexural strength and elastic modulus ($p<0.05$). Post-hoc LSD analysis revealed significant differences among groups for both flexural strength and elastic modulus ($p<0.05$), with several exceptions in specific comparisons within the EDTA and Ultra Clean groups, as well as between Ultra Clean manual agitation subgroup and the 0.2% chitosan nanoparticle subgroup ($p>0.05$). **Conclusion:** The highest flexural strength and elastic modulus values were observed in the group treated with 0.2% chitosan nanoparticles combined with sonic agitation, while the lowest values were found in the 17% EDTA group using manual agitation.*

Keywords

final irrigation, irrigation technique, flexural strength, modulus of elasticity, root canal dentin.

Efek jenis bahan irigasi akhir dan teknik irigasi terhadap kekuatan fleksural dan modulus elastisitas dentin saluran akar: studi eksperimental in vitro

ABSTRAK

***Pendahuluan**: Irigasi saluran akar berfungsi membersihkan debris, melumasi saluran, melarutkan jaringan organik dan anorganik, serta mencegah terbentuknya smear layer. Namun, sifat khelasi pada bahan irigasi akhir berpotensi mengikis dinding saluran akar dengan mengikat ion kalsium, yang dapat memengaruhi kekuatan fleksural dan modulus elastisitas dentin. Penelitian ini bertujuan untuk menganalisis pengaruh jenis bahan irigasi akhir dan teknik agitasi terhadap sifat mekanis dentin saluran akar akar. **Metode:** Empat puluh lima premolar mandibula di preparasi, dibagi menjadi tiga kelompok secara acak masing-masing kelompok terdiri dari 15 sampel. Kelompok 1 di irigasi dengan EDTA, kelompok 2 diirigasi dengan kitosan nanopartikel 0,2% dan kelompok 3 di irigasi dengan Ultra clean. Masing-masing kelompok dibagi lagi menjadi 3 kelompok dan setiap kelompok terdiri 5 sampel. Kelompok A digitasi manual, kelompok B sonik, dan kelompok C ultrasonik. Setelah itu dilakukan uji =kekuatan fleksural dan modulus elastisitas dengan Universal Testing Machine (UTM). **Hasil**: Uji ANAVA Bahan irigasi akhir, teknik irigasi, dan interaksi keduanya berpengaruh signifikan terhadap kekuatan fleksural dan modulus elastisitas ($p<0,05$). Hasil Post-Hoc LSD menunjukkan perbedaan signifikan antarkelompok untuk kekuatan fleksural dan modulus elastisitas dentin ($p<0,05$), dengan beberapa pengecualian pada perbandingan metode tertentu dalam kelompok EDTA 17% dan Ultra Clean, serta antara Ultra Clean manual dan kitosan nanopartikel 0,2% ($p>0,05$). **Simpulan**: Nilai kekuatan fleksural dan modulus elastisitas tertinggi pada penggunaan kitosan nanopartikel 0,2% dengan teknik agitasi sonik serta terendah pada penggunaan EDTA 17% dengan teknik agitasi manual.*

Kata kunci

bahan irigasi akhir, teknik irigasi, kekuatan fleksural, modulus elastisitas.

INTRODUCTION

A successful root canal treatment involves biomechanical preparation (cleaning and shaping), a method of root canal debridement using chemical agents such as irrigating solutions.[1] In addition, this treatment helps preserve the tooth within the oral cavity, and maintain masticatory function.[2] The key success of root canal treatment is based on the application of the endodontic triad principles, including biomechanical preparation (cleaning and shaping), root canal disinfection using chemical agents such as irrigating solutions, and root canal obturation using filling materials and sealers.[3]

The instrumentation process during biomechanical preparation produces debris that adheres to the root canal wall as smear layer. This smear layer consists of organic and inorganic tissue that can block the dentinal tubules, inhibiting the penetration of irrigating solution and sealers during obturation.[4] Therefore, smear layer removal is an essential step in ensuring the success of root canal treatment.[5]

One of the most commonly used irrigating solution in clinical practice is sodium hypochlorite (NaOCl). At concentrations ranging from 2.5-5.0%, NaOCl is capable in dissolving organic tissue and eliminating microorganisms within the root canal system.[6] However, this solution has limitations, as it us unable to dissolve inorganic components, allowing the smear layer to remain attached to the root canal walls. Therefore, the 17% *Ethylenediaminetetraacetic acid* (EDTA) is an alternative irrigating agent to address this issue.[7]

Ethylenediaminetetraacetic acid (EDTA) is capable of dissolving inorganic tissue and remove the smear layer that obstructs dental tubules. Unfortunately, prolonged use of 17% EDTA may lead to dental corrosion, which eventually reduce both the flexural strength and the elastic modulus of dentin.[7] Therefore, studies on safer alternative final irrigating solutions continues to be explored.

Ultra Clean is also one of the final irrigating solutions commonly used in clinical practice. This solution contains benzalkonium chloride (BAK), sodium citrate, and steviol glycosides.[8] The sodium citrate component found in Ultra Clean acts as a chelating agent that can help in smear layer removal without causing significant dentin erosion. In addition, steviol glycosides provide flavor to the irrigation solution, thereby enhancing patient comfort.[9]

Recent studies have also showed the potential use of chitosan as a final irrigation solution. Chitosan is a natural polysacharide obtained through the deacetylation of chitin derived from crustacean shells. Chitosan possesses chelating properties that enable this solution to effectively remove the smear layer.[10] Modification of chitosan into nanoparticles has been shown to enhance its effectiveness as an irrigating solutions due to its small particle size, allowing better penetration into dentinal tubules and complex areas of the root canal system.[6]

Studies using 0.2 % chitosan nanoparticles have demonstrated effectiveness comparable to that of 17% EDTA in opening dentinal tubules without causing excessive dentin erosion.[11] This finding suggests that chitosan nanoparticles are a promising irrigating agent with minimal risk of adverse effects. In addition to the selection of irrigating components, irrigation techniques also play an important role in the distribution of the solutions within root canal system. The techniques of irrigation can be classified into manual methods (manual dynamic agitation) and machine-assisted method (sonic and ultrasonic).[12] The manual irrigation method is frequently insufficient for cleaning the entire root canal system, particularly in the apical region, which is difficult to access.

Both sonic and ultrasonic agitation methods allow more effective activation of irrigating solutions. Sonic irrigation operates at frequencies ranging from 1–6 kHz using polymer tips that do not damage the root canal walls.[2] In contrast, ultrasonic irrigation technique operates frequencies between 25 to 30 kHz with vibrating metal files, generating acoustic waves and cavitation phenomena that enhance the distribution of irrigating solution throughout the root canal system.[13,14]

Agitation techniques are commonly used to promote smear layer removal and improve the distribution of irrigating solution throughout the root canal system.[15] Previous studies have reported that the ultrasonic techniques are significantly more effective in removing the smear layer compared with manual techniques.[2] The biomechanical properties of dentin, including flexural strength and elastic modulus, are important parameters that may be affected by both the irrigating agents and the techniques used.[16] Flexural strength measures the ability of dentin to resist combined stresses, whereas elastic modulus reflects the stiffness of dentin.[17] Root canal irrigation is vital for removing debris and preventing smear layer; however, the chelating properties of these irrigating agents can lead to the depletion of calcium from the dentin. This study aimed to analyzed the effect of different final irrigating solutions and agitations techniques on the flexural and the elastic modulus of root canal dentin.

METHODS

This study is an in vitro laboratory experiment. The 0.2% chitosan nanoparticles solution was prepared by dissolving chitosan powder in 1% acetic acid 1%, which was the stirred using magnetic stirrer for 1 hour at room temperature. A tripolyphosphate (TPP) solutions, at a volume ratio of 5:1 was the added gradually under continuous stirring for 1 hour. To obtain a 0,2% chitosan nanoparticle irrigating solution, the ionically gelated chitosan was dissolved in 1% acetic acid. Subsequently, 100 mL of the solution was stirred at 500 rpm for 30 minutes until a homogenous solution was achieved.[18] The concentration was calculated using the dilution formula = $M_1 \times V_1 = M_2 \times V_2$ (M_1: initial concentration, V_1: initial Volume/mass, M_2: final concentration, and V_2: final volume/mass)

The preparation phase began with determining the sample size using Federer's formula: $(t-1)\ (n-1) \geq 15$, where $t = 9$ treatment groups. Based on this calculation, the minimum required sample size was $n \geq 2.875$. Therefore, five samples were assigned to each group to satisfy the statistical requirements. A total of 45 mandibular premolars were included in the study and randomly allocated into three groups according to the final irrigating solution used: 17% EDTA, Ultra Clean, and 0.2% chitosan nanoparticles ($n = 15$ per group). Each group was further divided into three subgroups based on the agitation technique employed (manual, sonic, and ultrasonic), with five samples assigned to each subgroup.

Subsequently, the forty-five mandibular premolars that met the inclusion criteria were examined radiographically to confirm the presence of a single, straight root canal. The tooth specimens were rinsed with sterile distilled water and cleaned of debris and residual tissue, then stored at room temperature in a sealed container containing saline solution to prevent dehydration. The crowns of all forty-five teeth were sectioned using a diamond disc bur mounted on a low-speed handpiece to obtain a standardized root length of 13 mm (Figure 1).

After all teeth had been sectioned, they were embedded in wax boxes, with the apical end of each root sealed using red utility wax. The specimens were then randomly assigned to nine groups, with five teeth in each group. Each group was placed in a container lined with gauze moistened with saline solution and subsequently stored in an incubator at 37°C for 14 days.[18]

A B

Figure 1. Sectioning of 45 tooth specimens at the crown portion. (A) Tooth sectioning procedure using a diamond bur; (B) Sectioned tooth specimen with a standardized length of 13 mm.

Root canal preparation was performed using an endomotor and the crown-down technique with rotary files, following the prescribed file sequence and working length, and was completed using an F3 file. A size #10 K-file was inserted into the root canal until the apical region, and the working length (WL) was established at 1 mm short of the apical foramen.[18] The canal was irrigated with 3 mL of 2.5% sodium hypochlorite (NaOCl) followed by saline solution after each file change.

Following root canal preparation, the tooth samples were randomly assigned to their respective groups. Each sample was given an identification number; these numbers were then placed into a closed container and drawn blindly to allocate the samples into the final irrigation treatment groups: 17% EDTA, Ultra Clean, and 0.2% chitosan nanoparticles (n=15 per group). Each sample was irrigated with 5 ml of solution using a single-vented needle positioned 1 mm short of the apex. In subgroup A, final irrigation was performed using a manual irrigation technique (n=5); in subgroup B, final irrigation included sonic agitation for 30 seconds (n=5); and in subgroup C, final irrigation was accompanied by ultrasonic agitation activation for 30 seconds (n=5). Finally, all samples were rinsed with 3 ml of saline for 1 minute.

A B C

Figure 2. Final root canal irrigation procedures. (A) Manual agitation technique; (B) Sonic agitation technique; (C) Ultrasonic agitation technique

To prepare the specimens for analysis, the treated teeth were sectioned into rectangular dentin block (8 mm in length, 2 mm in width, and 3 mm) using a diamond disc bur on a low-speed handpiece. These blocks were then polished with No. 1500 silicon carbide grit (Multi-Tech Products, Anaheim, CA, USA) and subsequently examined under a light microscope at 1.6× and 2.5× magnifications to check for the presence of any cracks.

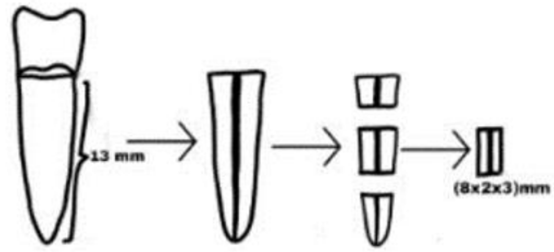

Figure 3. Schematic illustration of the specimen sectioning method used in this study

Flexural strength and modulus of elasticity testing was conducted at the Materials Laboratory, Department of Mechanical Engineering, Faculty of Engineering, Universitas Negeri Yogyakarta. The testing was performed using a three-point bending test on a Universal Testing Machine (UTM), which utilizes three contact points: two lower points to support the specimen and one upper point to apply the load to the center of the sample's top surface.

The load was applied at a crosshead speed of 1 mm/min. Each sample was positioned across the two supports, spanning a distance of 6 mm. The flexural strength and modulus of elasticity were calculated using the standard formulas: = 3 X (load) X (length)/2 X (width) X (thickness)^2. Deformas = load) X (length)^3/4X (E) X (width) X (thickness).[21]

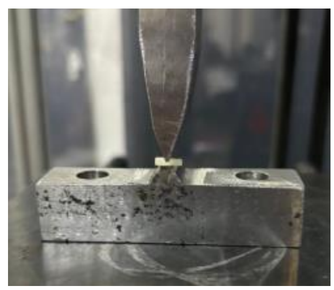

Figure 4. *Three-point bending* test performed on the specimens.

The data obtained from this study were ratio-scale data and were first subjected to the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. Once the assumption of normality and homogeneity were met, the data were then analyzed using a two-way ANOVA at a 5% significance level. Subsequently, the *Least Significant Difference* (LSD) post hoc test was applied to groups exhibiting significant differences. Statistical analysis was performed using SPSS software version 26.

RESULTS

This in vitro study used 45 mandibular premolar samples to evaluate the effects of three final irrigants (17% EDTA, Ultra Clean, and 0.2% Chitosan nanoparticles) and three different irrigation techniques (manual, sonic dan ultrasonic agitation) on the flexural strength and modulus of elasticity of dentin. The mean values for both flexural strength and modulus of elasticity across the different final irrigants and irrigation techniques are presented in bar graph (Figure 5 and Figure 6).

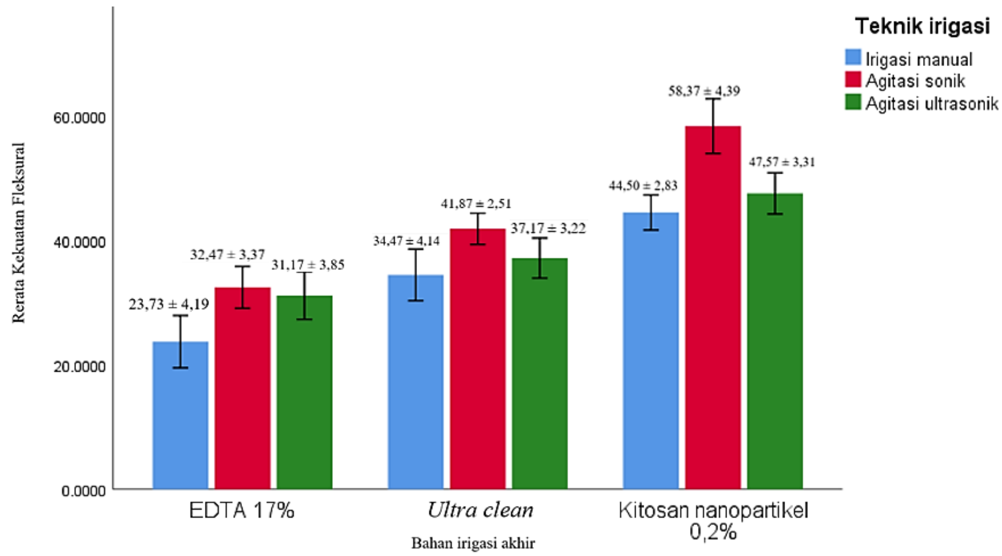

Figure 5. Mean values and standard deviations of root canal dentin flexural strength (MPa) according to the final irrigation solutions and irrigation techniques

The study demonstrates that the highest mean flexural strength was observed in the 0.2% Chitosan nanoparticle group combined with sonic agitation, whereas the lowest mean was found in the 17% EDTA using the manual irrigation technique.

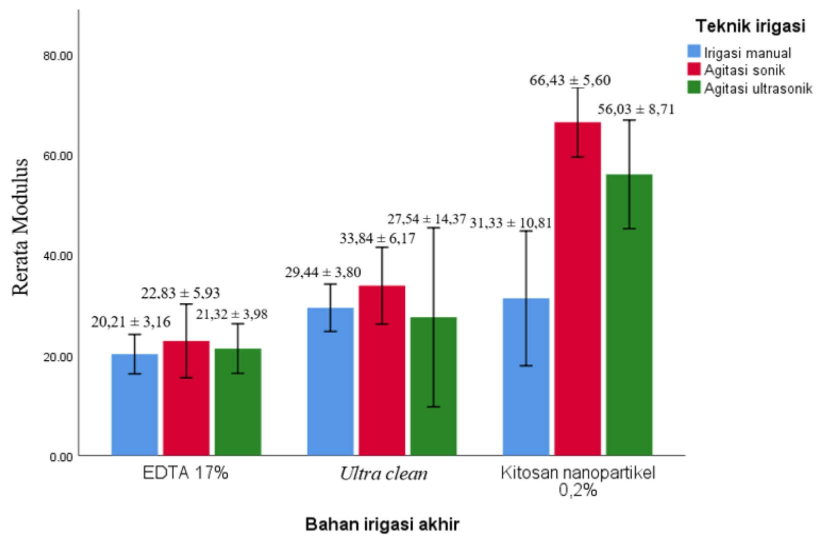

Figure 6. Mean values and standard deviations of the modulus of elasticity of root canal dentin (GPa) according to the final irrigation solutions and irrigation techniques.

Similarly, the highest mean value of elasticity modulus was observed in the 0.2% Chitosan nanoparticle group combined with the sonic irrigation technique, whereas the lowest mean value was found in the EDTA 17% final irrigant group using manual irrigation. The obtained data were then subjected to the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. The results of the Shapiro-Wilk normality test for both flexural strength and modulus of elasticity are presented in Table 1.

Table 1. Results of the Shapiro–Wilk and Levene's Tests for the Effects of Final Irrigation Solutions and Irrigation Techniques on the Flexural Strength and Modulus of Elasticity of Root Canal Dentin

Group	Nilai P Uji			
	***Shapiro Wilk* Flexural Strength**	***Shapiro Wilk* Modulus of Elasticity**	***Levene Test* Flexural Strength**	***Levene Test* Modulus of Elasticity**
EDTA, Manual	0.360*	0.198*	0.764*	0.200*
EDTA, Sonic	0.062*	0.166*		
EDTA, Ultrasonic	0.720*	0.174*		
Ultra Clean, Manual	0.397*	0.731*		
Ultra Clean, Sonic	0.423*	0.138*		
Ultra Clean, Ultrasonic	0.433*	0.086*		
Chitosan Nanoparticles, Manual	0.879*	0.944*		
Chitosan Nanoparticles, Sonic	0.201*	0.552*		
Chitosan Nanoparticles, Ultrasonic	0.329*	0.483*		

Note : * = significant at p>0.05

The normality test results for the flexural strength data in each group yielded p-values greater than 0.05 ($p > 0.05$), indicating that the data were normally distributed. Levene's test for homogeneity of variance revealed a p-value of 0.764 ($p=0.764$), which means there was no significant difference in variance among the nine groups, confirming variance homogeneity. Since the data met the assumptions of normal distribution and homogeneity, a two-way ANOVA was deemed appropriate for further analysis.

Similarly, the normality test for the modulus of elasticity data showed that all groups obtained p-values greater than 0.05 ($p>0.05$), indicating a normal distribution. The homogeneity of variance analysis via Levene's test yielded a p-value of 0.200 ($p = 0.200$), indicating no significant difference in variance across the nine groups (homogenous variance). Consequently, because the experimental data met the criteria for parametric testing, a two-way ANOVA was conducted to analyze the mean differences between the groups, as presented in Table 2 and Table 3.

Table 2. Results of the Two-Way ANOVA for the Effects of Final Irrigation Solutions and Irrigation Techniques on the Flexural Strength of Root Canal Dentin

Variable	Sum of Squares	DF	Mean Square	F	p
Final irrigation solution	3346.90	2	1673.45	130.089	0.001*
Irrigation technique	753.60	2	376.80	29.291	0.001*
Irrigation solution × Irrigation technique	139.20	4	34.80	2.705	0.046*

Note : * = statistically significant (p<0.05)

Two-way ANOVA test revealed that both the final irrigant and the irrigation technique had a significant effect on the flexural strength ($p < 0.05$). Furthermore, a significant interaction was observed between the final irrigant and the irrigation technique regarding their effect on flexural strength ($p < 0.05$).

Table 3. Results of the Two-Way ANOVA for the Effects of Final Irrigation Solutions and Irrigation Techniques on the Modulus of Elasticity of Root Canal Dentin

Variable	Sum of Squares	DF	Mean Square	F	p
Final irrigation solution	0.007	2	0.003	86.15	0.000*
Irrigation technique	0.002	2	0.001	19.55	0.000*
Irrigation solution × Irrigation technique	0.002	4	0.000	11.07	0.000*

Note : * = statistically significant (p<0.05)

Similarly, the two-way ANOVA results for the modulus of elasticity demonstrated that both the final irrigant and the irrigation technique showed a significant influence ($p < 0.05$). A significant interaction effect between the final irrigant and the irrigation technique on the modulus of elasticity was also detected ($p < 0.05$). To determine specific group differences, the Least Significant Difference (LSD) post hoc test was subsequently performed; the results are presented in Table 4 for flexural strength and Table 5 for the modulus of elasticity.

Table 4. Results of the Least Significant Difference (LSD) Post Hoc Test for the Effects of Final Irrigation Solutions and Irrigation Techniques on the Flexural Strength of Root Canal Dentin

(I) Group	(J) Group	Mean Difference (I-J)	p
EDTA, Manual	EDTA, Sonic	-8.73	0.000*
	EDTA, Ultrasonic	-7.43	0.002*
	Ultra clean, Manual	-10.73	0.000*
	Ultra clean, Sonic	-18.13	0.000*
	Ultra clean, Ultrasonic	-12.20	0.000*
	Chitosan Nanoparticles, Manual	-20.76	0.000*
	Chitosan Nanoparticles, Sonic	-34.63	0.000*
	Chitosan Nanoparticles, Ultrasonic	-23.70	0.000*
EDTA, Sonic	EDTA, Ultrasonic	1.30	0.570
	Ultra clean, Manual	-2.00	0.384
	Ultra clean, Sonic	-9.40	0.000*
	Ultra clean, Ultrasonic	-4.70	0.045*
	Chitosan Nanoparticles, Manual	-12.03	0.000*
	Chitosan Nanoparticles, Sonic	-25.90	0.000*
	Chitosan Nanoparticles, Ultrasonic	-15.10	0.000*
EDTA, Ultrasonic	*Ultra clean,* Manual	-3.30	0.154
	Ultra clean, Sonic	-10.70	0.000*
	Ultra clean, Ultrasonic	-6.00	0.012*
	Chitosan Nanoparticles, Manual	-13.30	0.000*
	Chitosan Nanoparticles, Sonic	-27.20	0.000*
	Chitosan Nanoparticles, Ultrasonic	-16.40	0.000*
Ultra clean, Manual	*Ultra clean,* Sonic	-7.40	0.002*
	Ultra clean, Ultrasonic	-4.70	0.045*
	Chitosan Nanoparticles, Manual	-10.33	0.000*
	Chitosan Nanoparticles, Sonic	-23.90	0.000*
	Chitosan Nanoparticles, Ultrasonic	-13.10	0.000*
Ultra clean, Sonic	*Ultra clean,* Ultrasonic	4.70	0.045*
	Chitosan Nanoparticles, Manual	-2.26	0.002*
	Chitosan Nanoparticles, Sonic	-16.50	0.000*
	Chitosan Nanoparticles, Ultrasonic	-5.70	0.017*
Ultra clean, Ultrasonic	Chitosan Nanoparticles, Manual	-7.33	0.003*
	Chitosan Nanoparticles, Sonic	-21.20	0.000*
	Chitosan Nanoparticles, Ultrasonic	-10.40	0.000*
Chitosan Nanoparticles, Manual	Chitosan Nanoparticles, Sonic	-13.86	0.000*
	Chitosan Nanoparticles, Ultrasonic	-3.06	0.004*
Chitosan Nanoparticles, Sonic	Chitosan Nanoparticles, Ultrasonic	10.80	0.000*

Note : * = statistically significant ($p<0.05$)

The post-hoc LSD analysis for flexural strength presented in Table 4 revealed significant differences among most sample groups ($p < 0.05$). The only exceptions were found between the 17% EDTA with sonic agitation and 17% EDTA with ultrasonic agitation groups ($p>0.05$), between 17% EDTA with sonic agitation and Ultra Clean with manual irrigation ($p>0.05$), and between 17% EDTA with ultrasonic agitation and Ultra Clean with manual irrigation ($p>0.05$).

Table 5. Results of the Least Significant Difference (LSD) Post Hoc Test for the Effects of Final Irrigation Solutions and Irrigation Techniques on the Modulus of Elasticity of Root Canal Dentin

(I) Group	(J) Group	Mean Difference (I-J)	p
EDTA, Manual	EDTA, Sonic	-0.00	0.501
	EDTA, Ultrasonic	-0.00	0.765
	Ultra clean, Manual	-0.00	0.025*
	Ultra clean, Sonic	-0.01	0.001*
	Ultra clean, Ultrasonic	-0.01	0.003*
	Chitosan Nanoparticles, Manual	-0.01	0.008*
	Chitosan Nanoparticles, Sonic	-0.04	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.03	0.000*
EDTA, Sonic	EDTA, Ultrasonic	0.00	0.707
	Ultra clean, Manual	-0.00	0.106
	Ultra clean, Sonic	-0.01	0.009*
	Ultra clean, Ultrasonic	-0.00	0.018*
	Chitosan Nanoparticles, Manual	-0.00	0.040*
	Chitosan Nanoparticles, Sonic	-0.04	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.03	0.000*
EDTA, Ultrasonic	*Ultra clean,* Manual	-0.00	0.049*
	Ultra clean, Sonic	-0.01	0.003*
	Ultra clean, Ultrasonic	-0.01	0.007*
	Chitosan Nanoparticles, Manual	-0.01	0.017*
	Chitosan Nanoparticles, Sonic	-0.04	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.03	0.000*
Ultra clean, Manual	*Ultra clean,* Sonic	-0.00	0.278
	Ultra clean, Ultrasonic	-0.00	0.414
	Chitosan Nanoparticles, Manual	-0.00	0.640
	Chitosan Nanoparticles, Sonic	-0.03	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.02	0.000*
Ultra clean, Sonic	*Ultra clean,* Ultrasonic	0.00	0.785
	Chitosan Nanoparticles, Manual	0.00	0.003*
	Chitosan Nanoparticles, Sonic	-0.03	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.02	0.000*
Ultra clean, Ultrasonic	Chitosan Nanoparticles, Manual	0.00	0.002*
	Chitosan Nanoparticles, Sonic	-0.03	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.02	0.000*
Chitosan Nanoparticles, Manual	Chitosan Nanoparticles, Sonic	-0.03	0.000*
	Chitosan Nanoparticles, Ultrasonic	-0.02	0.000*
Chitosan Nanoparticles, Sonic	Chitosan Nanoparticles, Ultrasonic	0.01	0.013*

Note : * = statistically significant ($p<0.05$)

For the modulus of elasticity, the post-hoc LSD results in Table 5 also demonstrated significant differences across the sample groups ($p<0.05$), except for the comparisons between 17% EDTA manual and 17% EDTA sonic ($p>0.05$); 17% EDTA manual and 17% EDTA ultrasonic ($p>0.05$); 17% EDTA ultrasonic and 17% EDTA sonic ($p>0.05$); Ultra Clean manual and Ultra Clean sonic ($p>0.05$); Ultra Clean ultrasonic and Ultra Clean sonic ($p>0.05$); Ultra Clean ultrasonic and Ultra Clean manual ($p>0.05$); as well as between Ultra Clean manual and 0.2% chitosan nanoparticles with manual irrigation ($p>0.05$).

DISCUSSION

The interaction between final irrigants and irrigation techniques contributes to the effective elimination of the smear layer from root canal dentin, which subsequently enhances its flexural strength and modulus of elasticity. The two-way ANOVA results demonstrated significant differences ($p<0.05$) regarding the choice of final irrigant, irrigation technique, and their interaction on the mechanical parameters of root canal dentin.

Smear layer removal following the use of sodium hypochlorite becomes more effective when combined with an appropriate final irrigant and irrigation technique. The irrigation techniques evaluated in this study included manual, sonic, and ultrasonic agitation. The two-way ANOVA confirmed that both the type of final irrigant and the irrigation technique exerted a significant influence on the flexural strength and modulus of elasticity of root canal dentin ($p<0.05$).

Combining 0.2% chitosan nanoparticles with sonic agitation yielded higher flexural strength and modulus of elasticity compared to 17% EDTA and Ultra Clean. Unlike 17% EDTA and Ultra Clean, which exert strong chelating effects, chitosan possesses only a single amine group that interacts with metal ions, resulting in a more controlled chelation process. Consequently, this prevents excessive binding of dentin minerals, thereby preserving the structural integrity and mechanical properties of the dentin. The mechanism of action of chitosan—operating via the bridge model and the single amine group model—allows for a more optimal interaction with metal ions.[22] Furthermore, the minuscule size of chitosan nanoparticles enables deeper penetration into the dentinal tubules, enhancing cleaning efficacy compared to macro-chitosan. Modifying chitosan into nanoparticles aims to improve its absorption and diffusion during final irrigation.[22] The removal of the smear layer by chitosan occurs through adsorption, ion exchange, and chelation. Additionally, the hydrophilic nature of chitosan enhances the adsorption of the solution onto the dentin, facilitating ionic interactions with calcium for optimal chelation.[24]

The 0.2% chitosan nanoparticles demonstrated superiority in preserving the flexural strength and modulus of elasticity of dentin due to a more controlled chelating effect compared to 17% EDTA and citric acid, which can induce dentin erosion. Previous studies have indicated that chitosan can enhance the mechanical properties of dentin by interacting with dentinal collagen. This interaction occurs through the formation of hydrogen bonds between the -OH, -NH2, and -C=O groups in collagen and the -OH and -NH2 groups in chitosan.[23] Chitosan also maintains covalent bonds with dentinal collagen, thereby contributing to the remineralization of demineralized dentin.[26] Previous studies have indicated that chitosan can enhance the mechanical properties of dentin.[27] Chitosan can form covalent bonds with dentinal collagen, thereby facilitating the remineralization of demineralized dentin.[24]

The 17% EDTA solution exhibits a stronger chelating capacity compared to Ultra Clean and chitosan. The abundance of hydroxyl and amine groups in EDTA allows for greater binding of calcium ions.,[25] which leads to a reduction in the inorganic structure of the dentin, subsequently decreasing its flexural strength and modulus of elasticity. The chemical structure of EDTA, featuring four carboxyl groups and two amine groups, forms a highly stable chelate complex.[27] Thbayh *et al.*[26] reported that EDTA, as a hexadentate chelator, can bind up to six coordination sites on a metal ion simultaneously, making it a highly effective agent for removing both the smear layer and dentin minerals.

Ultra Clean, which contains citric acid in sodium citrate, acts as chelating agent with a near-neutral pH, making it more biocompatible and effective for dentin decalcification without causing excessive erosion.[29] However, information regarding the exact percentage of sodium citrate in Ultra Clean remains limited, which may affect its efficacy during root canal irrigation.[30] explains that citric acid undergoes three stages of ionization, allowing multiple hydroxyl groups to bind with metal ions within the smear layer and root canal dentin. Furthermore, Sarkees and Maarrawi,[28] stated that citric acid in 10% sodium citrate exhibits a near-neutral pH, good biocompatibility, and high efficiency in dentin decalcification. The demineralizing effect of sodium citrate on dentin tends to be lower than that of other demineralizing agents, such as pure citric acid and EDTA, due to its near-neutral pH. Nevertheless, specific formulation details regarding the sodium citrate percentage in Ultra Clean are not available.

The post-hoc LSD test indicated that the sonic agitation technique yielded higher flexural strength and modulus of elasticity compared to both manual and ultrasonic agitation. This technique facilitates a more uniform distribution of the irrigating solution along the root canal walls, effectively reaching the apical region. Sonic agitation operates at a lower frequency (1–6 kHz) than ultrasonic agitation (25–30 kHz), thereby enabling thorough flushing of the canal without inducing dentin erosion.[5,12] Conversely, manual agitation resulted in the lowest flexural strength and modulus of elasticity. This outcome is attributed to the absence of vibrations required to optimize solution distribution, which leads to suboptimal cleaning and the potential accumulation of chelating agents, subsequently compromising the mechanical properties of the dentin.[29]

This study demonstrates that the combination of 0.2% chitosan nanoparticles with the sonic agitation technique yields the most optimal results in enhancing the flexural strength and modulus of elasticity of root canal dentin. This specific combination facilitates deeper penetration of the solution without causing damage to the root canal walls, thereby reducing the risk of calcium and phosphorus (Ca/P) mineral loss in the dentin.[30]

Nevertheless, this study has several limitations, including variations in the baseline Ca/P conditions of the dentin, structural differences in collagen and dentinal tubules, and the inherent difficulty of obtaining completely uniform tooth samples. Furthermore, dentin anisotropy is a contributing factor in influencing the testing outcomes as the mechanical properties of dentin are highly dependent on the direction of the applied load.[20] Consequently, further investigations, involving both *in vitro* and *in vivo* studies, are required to comprehensively evaluate the efficacy of 0.2% chitosan nanoparticles combined with sonic irrigation techniques on the flexural strength and modulus of elasticity of root canal dentin.

CONCLUSION

The use of 0.2% chitosan nanoparticles demonstrated higher flexural strength and modulus of elasticity values for root canal dentin compared to 17% EDTA and Ultra Clean. Regarding the irrigation techniques, the sonic agitation technique yielded higher values than both manual and ultrasonic techniques. Furthermore, the combination of 0.2% chitosan nanoparticles with sonic agitation produced the highest values under laboratory testing conditions. These findings imply that chitosan holds potential as a safer alternative irrigant to EDTA by minimizing the risk of post-treatment tooth fracture, although further clinical validation remains necessary.

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REFERENCES

1. Permatasari R, Radya M, Kamal P. Perbedaan penggunaan sealer berbahan dasar resin dan mineral trioxide aggregate terhadap kerapatan obturasi saluran akar. MDERJ. 2022;2(1):1–7.
2. Hargreaves KM, Berman LH. *Cohen's pathways of the pulp expert consult*. 11th ed. St. Louis: Elsevier; 2016. p. 854.

3. Widyastuti A, Santosa P. Perawatan saluran akar dengan instrumen putar dan restorasi resin komposit penguat fiber. Maj Ked Gi Klin UGM. 2018;4(1):9–19.
4. Mohammadi Z, Shalavi S, Yaripour S, Kinoshita JI, Manabe A, Kobayashi M, et al. Smear layer removing ability of root canal irrigation solutions: a review. J Contemp Dent Pract. 2019;20(3):395–402. https://doi.org/10.5005/jp-journals-10024-2528
5. Ali A, Bhosale A, Pawar S, Kakti A, Bichpuriya A, Agwan MA. Current trends in root canal irrigation. Cureus. 2022;14(5):1–8. https://doi.org/10.7759/cureus.24833
6. Ratih DN, Sari NI, Santosa P, Kaswati NMN. Time-dependent effect of chitosan nanoparticles as final irrigation on the apical sealing ability and push-out bond strength of root canal obturation. Int J Dent. 2020;2020:1–7. https://doi.org/10.1155/2020/8887593
7. Hosseini S, Kassaee MZ, Elahi SH, Bolhari B. A new nano-chitosan irrigant with superior smear layer removal and penetration. Nanochem Res. 2016;1(2):150–6.
8. Titato PCG, Zancan RF, Pedrinha VF, de Andrade FB, Vivan RR, Duarte MAH. Influence of EDTA and its association with benzalkonium chloride on *Enterococcus faecalis* adhesion to dentin. Int J Odontostomat. 2020;14(4):632–8. https://doi.org/10.4067/s0718-381x2020000400632
9. Shinde MR, Winnier J. Health benefits and application of *Stevia rebaudiana* Bertoni in dentistry. J Drug Deliv Ther. 2020;10:271–4. https://doi.org/10.22270/jddt.v10i4-s.4285
10. Nugroho A, Kusumo H, Mulyawati E, Ratih DN. Apical sealing ability of epoxy resin sealer after application of final irrigation using EDTA and chitosan nanoparticle with different contact time. MDERJ. 2020;4(1):6–11.
11. Ratih DN, Enggardipta RA, Kartikaningtyas AT. The effect of chitosan nanoparticle as a final irrigation solution on the smear layer removal, micro-hardness and surface roughness of root canal dentin. Open Dent J. 2020;14(1):19–26. https://doi.org/10.2174/1874210602014010019
12. Gopikrishna V. *Grossman's endodontic practice*. 14th ed. New Delhi: Wolters Kluwer; 2021. p. 152, 287–298.
13. Tashkandi N, Alghamdi F. Effect of chemical debridement and irrigant activation on endodontic treatment outcomes: an updated overview. Cureus. 2022. https://doi.org/10.7759/cureus.21525
14. Gadiya P, Girnar J, Dhatrak P, Ghorpade R. Review on modern day irrigation methods in endodontics. AIP Conf Proc. 2021;2358:1–9. https://doi.org/10.1063/5.0057945
15. Atav A, Zanza A, Gunes A, et al. Inovasi terkini dalam irigasi endodontik dan pengaruhnya terhadap penghilangan lapisan smear: sebuah studi ex-vivo. Clin Oral Investig. 2025;29:309. https://doi.org/10.1007/s00784-025-06387-1
16. Anusavice KJ. *Phillips science of dental materials*. 10th ed. Jakarta: EGC; 2003. p. 70–90.
17. Sakaguchi RL, Ferracane JL, Powers JM. *Craig's restorative dental materials*. 14th ed. Elsevier Inc.; 2018. p. 80–100.
18. Aydın ZU, Ozyurek T, Keskin B, Baran T. Effect of chitosan nanoparticle, QMix, and EDTA on TotalFill BC sealers' dentinal tubule penetration: a confocal laser scanning microscopy study. Odontology. 2019;107(1):64–71. https://doi.org/10.1007/s10266-018-0360-5
19. Kamble AB, Abraham S, Kakde DD, Shashidhar C, Mehta DL. Scanning electron microscopic evaluation of efficacy of 17% EDTA and chitosan for smear layer removal with ultrasonics: an in vitro study. Contemp Clin Dent. 2017;8(4):621–6. https://doi.org/10.4103/ccd.ccd_745_17
20. Agusmawanti P, Ratih DN, Purwanti N, Raharjo TJ. The Potential of QP3VH-Chitosan Peptide as Biomimetic Remineralization in Early Dental Caries Treatment: An In Vitro Study. Eur J Dent. 2024;18(4):1149–1156. https://doi.org/10.1055/s-0044-1782189
21. Permatasari R, Safitri A. Peran EDTA Sebagai Bahan Irigasi Pada Perawatan Saluran Akarnya. MDERJ. 2022;2(2):24-31. https://journal.moestopo.ac.id/index.php/mderj
22. Shashikumar G, Hinduja D, Mujeeb A, Raghu KN, Ashwini KS. Comparative evaluation of smear layer removal after the use of NaOCl with 17% EDTA versus silver citrate as root canal irrigant: a SEM study. Eur J Pharm Med Res. 2021;8(8):659–62.
23. 23. Namirah I, Pawitan JA. Collagen and chitosan as scaffold in tissue engineering. Internat Medic J. 2022;29(6):325–328.
24. Gronwald B, Kozłowska L, Kijak K, Lietz-Kijak D, Skomro P, Gronwald K, et al. Nanoparticles in dentistry—current literature review. Coatings. 2023;13(1):102. https://doi.org/10.3390/coatings13010102
25. D'Agosto G. Irrigants and irrigation protocols in endodontics: A literature review. Madrid: Universidad Europea de Madrid; 2023.
26. Thbayh DK, Palusiak M, Viskolcz B, Fiser B. Comparative study of the antioxidant capability of EDTA and Irganox. Heliyon. 2023;9(5):e16064. https://doi.org/10.1016/j.heliyon.2023.e16064
27. Sarkees M, Maarrawi K. Chitosan: a natural substitute of EDTA solution for final irrigation in endodontics treatment. Niger J Clin Pract. 2020;23(5):697–703. https://doi.org/10.4103/njcp.njcp_587_19
28. Muslov SA, Lisovenko DS. Elastic anisotropy of dentin and enamel. Lett Mater. 2018;8(3):288–93. https://doi.org/10.1088/1757-899X/441/1/012032
29. Mathew DM, Durvasulu A, Shanmugam S, Pradeep Kumar AR. Eur Endod J. 2023;8:72–78.
30. Tanumihardja M, Rehatta Y, Wahyuni S, Samad R, Mooduto L, Wahid S. Reduction of bacterial after irrigation with 6% NaOCl, and continued irrigation with 17% EDTA and 2% CHX during endodontic treatment of chronic apical periodontitis. Int J Pharm Res. 2020;Suppl 1:2540–2545. https://doi.org/10.31838/ijpr/2020.SP1.372