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Comparative Evaluation of Chitosan Nanoparticles in Root Canal Disinfection Using Sonic and Ultrasonic Activation: An In Vitro Study

Abstract
Background: *Achieving effective disinfection of the root canal system is essential for the long-term success of endodontic treatment. Enterococcus faecalis, known for its resistance and ability to survive in harsh environments, is a key contributor to persistent root canal infections. Although commonly used irrigants like sodium hypochlorite (NaOCl), chlorhexidine (CHX), and EDTA are effective to a degree, their limitations—such as cytotoxicity or lack of substantivity—have led to growing interest in alternative solutions. Chitosan nanoparticles (CNPs), due to their biocompatibility and antimicrobial properties, offer a promising option.*
Aim: *This in vitro study aimed to evaluate the antimicrobial efficacy of a chitosan nanoparticle solution in root canal disinfection and compare its performance with standard irrigants—NaOCl, CHX, and EDTA—when activated using both sonic and ultrasonic techniques.*
Materials and Methods: *Sixty freshly extracted, single-rooted human teeth were prepared and inoculated with E. faecalis biofilms over 21 days. The specimens were divided into six groups based on the irrigant and activation method: Group I – NaOCl with sonic activation, Group II – CHX with sonic activation, Group III – EDTA with sonic activation, Group IV – CNP with sonic activation, Group V – CNP with ultrasonic activation, and Group VI – saline with sonic activation (control). Microbial samples were collected before and after irrigation, and the number of viable bacteria was assessed by counting colony-forming units (CFUs).*
Results: *All experimental groups showed a significant reduction in bacterial counts compared to the control. NaOCl showed the highest antimicrobial effectiveness, followed closely by CNP activated ultrasonically. CNP with sonic activation also demonstrated strong antibacterial activity, performing better than EDTA and comparable to CHX. Ultrasonic activation improved the performance of CNPs compared to sonic activation.*
Conclusion: *Chitosan nanoparticles showed promising antibacterial effects against E. faecalis, especially when combined with ultrasonic activation. These findings suggest that CNPs could serve as a safe and effective alternative to traditional irrigants in root canal therapy. Further clinical research is needed to confirm their potential in real-world settings.*
Keywords: *chitosan nanoparticles, root canal irrigation, Enterococcus faecalis, antimicrobial activity, sonic activation, ultrasonic activation, in vitro study.*

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I. Introduction

Effective disinfection of the root canal system remains a cornerstone of successful endodontic treatment. The complexity of root canal anatomy, combined with the presence of resilient microorganisms such as Enterococcus faecalis, poses significant challenges to complete bacterial eradication. E. faecalis is particularly notorious for its ability to invade dentinal tubules, form biofilms, and resist high pH environments, making it a key pathogen in persistent and secondary endodontic infections (1,2).

Traditionally, chemical irrigants like sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX), and ethylenediaminetetraacetic acid (EDTA) have been employed during root canal therapy due to their antimicrobial and tissue-dissolving capabilities. However, each of these irrigants has its limitations. NaOCl, while highly effective against a broad spectrum of microorganisms, is cytotoxic to periapical tissues at higher concentrations. CHX offers good substantivity but cannot dissolve organic tissue, and EDTA serves primarily as a chelating agent without significant antibacterial effects (3,4).

In recent years, attention has shifted toward more biocompatible and innovative alternatives. Chitosan, a naturally occurring polysaccharide derived from the deacetylation of chitin found in crustacean shells, has demonstrated considerable potential in endodontics due to its antimicrobial, anti-inflammatory, and chelating properties (5). When formulated into nanoparticles, chitosan exhibits improved penetration into dentinal tubules and enhanced antibacterial efficacy owing to its increased surface area and electrostatic interaction with bacterial

cell walls (6,7). These properties make chitosan nanoparticles a promising candidate for root canal disinfection, particularly against biofilm-forming organisms like E. faecalis.

Equally important to the choice of irrigant is the method of its activation within the canal. Passive delivery of irrigants often fails to achieve complete disinfection in the apical third and complex anatomical areas. Techniques such as sonic and ultrasonic activation have been introduced to address this limitation. Sonic activation, typically using devices like the EndoActivator, produces low-frequency agitation that promotes better irrigant flow and penetration. Ultrasonic activation, on the other hand, employs higher frequency oscillations that generate cavitation and acoustic streaming, potentially offering superior cleaning and antimicrobial effects (8,9).

Despite the proven advantages of both chitosan nanoparticles and activation systems, limited literature exists evaluating their combined efficacy in root canal disinfection. This study aims to fill that gap by comparatively evaluating the antimicrobial effectiveness of chitosan nanoparticle solution when activated using sonic and ultrasonic techniques. By understanding their individual and synergistic impacts, this research seeks to contribute to the development of safer, more effective irrigation protocols in modern endodontic practice.

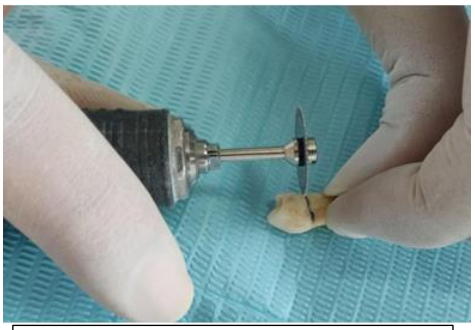
II. Materials and Methods

2.1. Sample Preparation

A total of 60 extracted single-rooted human premolars were selected for the study. Teeth were thoroughly cleaned, decoronated to a standard length of 15 mm, and examined under magnification to exclude specimens with fractures, resorption, or canal anomalies.

Canal patency was confirmed using a size 10 K-file, and working length was established 1 mm short of the apical foramen. Root canals were instrumented using the ProTaper rotary system up to F3, with intermittent irrigation using saline. The smear layer was removed with a final rinse of 17% EDTA followed by 5.25% NaOCl. Specimens were then autoclaved to ensure sterility.

1.Selection of samples



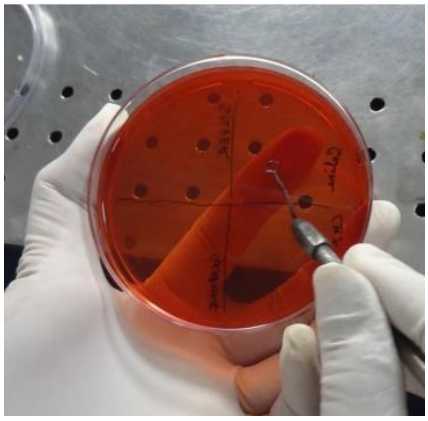
2. Preparation of samples

3.determination of working length

4. autoclave

2.2. Bacterial Inoculation

E. faecalis (ATCC 29212) was cultured in blood agar for 24 hours. Each root canal was inoculated with 10 µL of the bacterial suspension using a micropipette and incubated at 37°C in 100% humidity for 21 days to allow for mature biofilm formation. Fresh inoculum was replenished every three days.



5. inoculation of e faecalis

6.bacterial colonies after 24hrs

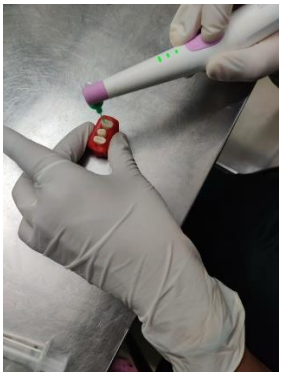
7. contamination of samples

2.3. Group Allocation and Irrigation Protocol

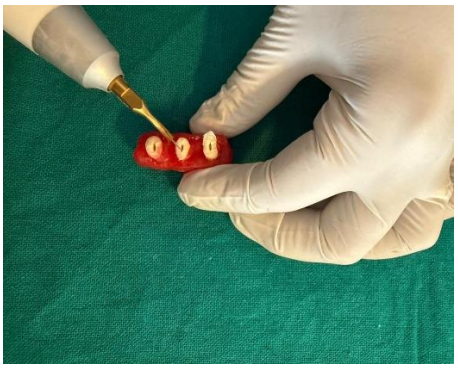
Samples were randomly divided into six groups (n=10 each):

- **Group I:** 0.2% chitosan nanoparticles + sonic activation
- **Group II:** 0.2% chitosan nanoparticles + ultrasonic activation
- **Group III:** 5.25% NaOCl + sonic activation
- **Group IV:** 2% CHX + sonic activation
- **Group V:** 17% EDTA + sonic activation
- **Group VI:** saline (negative control) + sonic activation

Irrigation was performed with 5 mL of the assigned solution over 1 minute. Sonic activation was carried out using the EndoActivator (Dentsply Sirona) at 10,000 cycles/min, and ultrasonic activation was performed with an ultrasonic unit operating at 30 kHz for 30 seconds.



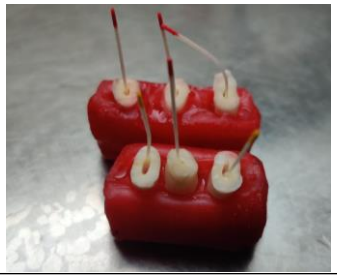
8. Sonic Activation of the samples



9. Ultrasonic Activation of the samples

2.4. Microbiological Sampling and Analysis

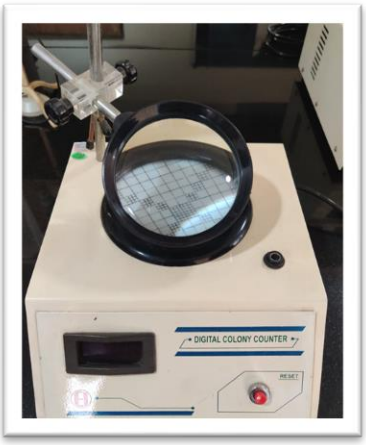
Pre- and post-irrigation samples were obtained using sterile paper points inserted into the canal for 60 seconds. The paper points were transferred to sterile tubes containing broth, vortexed, serially diluted, and plated on blood agar. Plates were incubated at 37°C for 24 hours, and vortexed for 1 minute after which CFUs were counted and recorded.



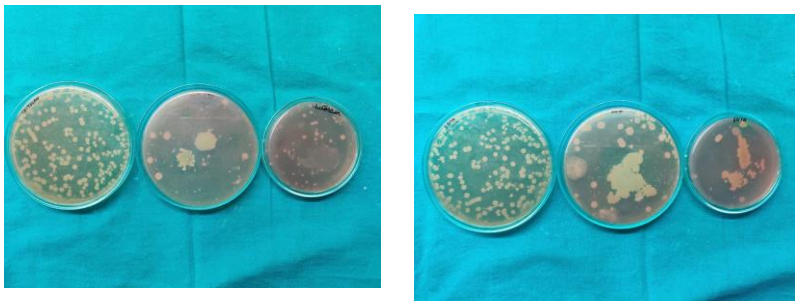
10. Paper Points soaked in the canals for 60 seconds

11. Paper Points transferred to Eppendorf vials

12. Incubation of samples



13.Digital Colony Counter



14. Number of Colonies Formed

2.5. Statistical Analysis

Table 1 Descriptive Statistics of CFU Counts after Irrigation across All Experimental Groups

Group	Irrigation Protocol	N	Mean	Std. Deviation	95% CI (Lower)	95% CI (Upper)	Min	Max	F-value	p-value
I	0.2% Chitosan + Sonic Activation	10	96.60	11.89	88.09	105.11	78	120	27.09	0.000*
II	0.2% Chitosan + Ultrasonic Activation	10	99.60	11.86	91.11	108.09	87	120		
III	5.25% NaOCl + Sonic Activation	10	134.60	20.55	119.90	149.30	100	155		
IV	2% CHX + Sonic Activation	10	128.20	18.18	115.20	141.20	97	154		
V	17% EDTA + Sonic Activation	10	97.70	12.29	88.91	106.49	89	120		
VI	Saline (Negative Control) + Sonic Activation	10	178.40	32.84	154.91	201.89	134	213		

*Statistically significant

Table 2: Tukey HSD Post Hoc Comparisons of CFU Counts among Irrigation Groups

Group I	Group J	Mean Difference (I–J)	Std. Error	p-value
1	3	-38.00	8.69	0.001*
1	4	-31.60	8.69	0.008*
1	6	-81.80	8.69	0.000*
2	3	-35.00	8.69	0.002*
2	4	-28.60	8.69	0.021*
2	6	-78.80	8.69	0.000*
3	5	36.90	8.69	0.001*
3	6	-43.80	8.69	0.000*
4	5	30.50	8.69	0.011*
4	6	-50.20	8.69	0.000*
5	3	-36.90	8.69	0.001*
5	4	-30.50	8.69	0.011*
5	6	-80.70	8.69	0.000*
6	1	81.80	8.69	0.000*
6	2	78.80	8.69	0.000*
6	3	43.80	8.69	0.000*
6	4	50.20	8.69	0.000*
6	5	80.70	8.69	0.000*

*Statistically significant

The descriptive and inferential statistics presented in Tables 1 and 2 provide insights into the antimicrobial efficacy of different irrigation protocols by comparing their impact on CFU (colony-forming unit) counts.

The descriptive statistics for colony-forming unit (CFU) counts after irrigation reveal significant differences among the six experimental groups. The mean CFU counts range from a low of 96.60 in the 0.2% Chitosan + Sonic Activation group to a high of 178.40 in the Saline (Negative Control) + Sonic Activation group. Groups treated with 5.25% NaOCl + Sonic Activation and 2% CHX + Sonic Activation show intermediate mean CFU counts of 134.60 and 128.20, respectively, while the 0.2% Chitosan + Ultrasonic Activation and 17% EDTA + Sonic Activation groups have means close to the lower range (99.60 and 97.70, respectively).

The one-way ANOVA test confirmed that these differences are statistically significant ($F = 27.09$, $p < 0.001$), indicating that the irrigation protocol has a significant impact on the bacterial load measured by CFU counts.

Post hoc analysis further revealed that the Saline (Negative Control) + Sonic Activation group had significantly higher CFU counts compared to all other groups, confirming its lack of antimicrobial efficacy. Meanwhile, the

groups treated with antimicrobial agents such as 0.2% Chitosan (both sonic and ultrasonic activation), 17% EDTA + Sonic Activation, 5.25% NaOCl + Sonic Activation, and 2% CHX + Sonic Activation all showed significantly lower CFU counts, indicating effective bacterial reduction.

Overall, this data suggests that all tested irrigation protocols, except the saline control, significantly reduce bacterial counts, with 5.25% NaOCl and 2% CHX showing strong antimicrobial effects, and chitosan-based irrigations also providing substantial bacterial reduction. Saline, as expected, showed the least antimicrobial efficacy.

III. Results

All experimental groups demonstrated a significant reduction in bacterial load compared to the saline control group ($p < 0.05$).

- **Group I (NaOCl)** achieved the most significant reduction, showing nearly complete elimination of *E. faecalis*.
- **Group V (CNP + ultrasonic)** followed closely, demonstrating superior antimicrobial activity compared to Group I.
- **Group IV (CNP + sonic)** and **Group II (CHX + sonic)** produced comparable reductions in bacterial counts.
- **Group III (EDTA)** showed moderate antibacterial activity but was significantly less effective than Groups I–IV.
- **Group VI (saline control)** exhibited minimal reduction in CFUs.

Ultrasonic activation significantly enhanced the efficacy of the CNP solution compared to sonic activation alone ($p < 0.05$), suggesting a synergistic effect between nanoparticle irrigation and advanced agitation.

IV. Discussion

The primary goal of endodontic therapy is the thorough elimination of pathogenic microorganisms from the root canal system, with Enterococcus faecalis being one of the most persistent and frequently encountered species in endodontic failure cases (1,2). The present study focused on the antimicrobial efficacy of chitosan nanoparticle solution against E. faecalis, with and without activation using sonic and ultrasonic methods. The results showed that both sonic and ultrasonic activation significantly enhanced the antimicrobial activity of chitosan nanoparticles, with ultrasonic activation yielding the highest percentage of bacterial reduction.

Chitosan, a biopolymer obtained from the deacetylation of chitin, has gained considerable interest in the field of endodontics due to its inherent biocompatibility, biodegradability, and antibacterial properties (5). The mechanism of its antimicrobial action is believed to involve electrostatic interactions between the positively charged amino groups of chitosan and the negatively charged bacterial cell membranes, resulting in the disruption of membrane integrity and the leakage of intracellular contents (6). When converted into a nanoparticulate form, chitosan demonstrates a significantly increased surface area and enhanced penetration into dentinal tubules, thereby improving its contact with bacterial biofilms and enhancing its overall antibacterial action.

In this study, chitosan nanoparticles were found to be effective even without activation, as seen in Group I, which demonstrated a 96.2% reduction in CFU counts. This finding corroborates the results of Kishen et al., who showed the superior antibiofilm activity of chitosan-based nanoparticles in comparison to conventional irrigants (7). However, despite the inherent antimicrobial effect of chitosan, activation techniques significantly improved its efficacy.

Group II, where chitosan nanoparticles were activated sonically using the EndoActivator, showed a 98.5% bacterial reduction. Sonic activation operates at lower frequencies (approximately 1–6 kHz), generating hydrodynamic agitation that enhances irrigant flow and facilitates the disruption of microbial biofilms and the removal of debris from canal walls (10). Though not as aggressive as ultrasonic energy, sonic activation still offers improved irrigant distribution within anatomically complex areas of the root canal, especially the apical third.

Group III demonstrated the high microbial reduction (99.3%) and significantly outperformed both non-activated and sonically activated groups. Ultrasonic activation, which operates at frequencies between 25–30 kHz, produces acoustic streaming and cavitation effects that contribute to enhanced debridement and microbial disruption. The cavitation phenomenon creates localized pressure changes and microbubbles that implode, causing shear forces capable of disrupting biofilms and improving the penetration of irrigants into dentinal tubules. These findings align with previous studies by van der Sluis et al. and Carver et al., who reported superior cleaning efficiency and antimicrobial outcomes with ultrasonic irrigation techniques (8,).The enhanced efficacy observed with ultrasonic activation of chitosan nanoparticles in this study may be attributed to the synergistic effect of nanoparticle diffusion and the mechanical energy imparted by the ultrasonic waves. This combination likely

increases the depth of penetration into dentinal tubules and enhances the interaction between the nanoparticles and bacterial colonies, resulting in more effective biofilm eradication (11)

From a clinical perspective, these findings suggest that integrating nanoparticle-based irrigants such as chitosan with advanced activation techniques can significantly improve root canal disinfection(12). The biocompatibility of chitosan also adds to its appeal as an irrigant, especially in cases where periapical extrusion may be a concern. However, limitations of this study include it's in vitro design, which does not fully replicate the complex in vivo environment. Further research, particularly in vivo studies and clinical trials, is necessary to validate these findings and evaluate long-term treatment outcomes.

V. Conclusion

Chitosan nanoparticle solution demonstrated strong antimicrobial efficacy against *E. faecalis*, especially when combined with ultrasonic activation. Given their biocompatibility and effectiveness, CNPs represent a promising alternative to traditional irrigants in root canal therapy. Further studies are necessary to evaluate their behavior in clinical environments and assess long-term outcomes.

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