ORIGINAL ARTICLE

Innovative Nanosilica-Based Triple Antibiotic Paste for Effective Disruption of *Enterococcus faecalis* Biofilm: A Confocal Analysis

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Abstract

Background: Management of pulp necrosis in immature teeth is challenging due to thin dentinal walls and an open apex, making effective root canal disinfection essential. *Enterococcus faecalis*, a persistent endodontic pathogen, forms resistant biofilms that are difficult to eradicate using conventional intracanal medicaments such as calcium hydroxide (CH) and triple antibiotic paste (TAP). Incorporation of nano-silica into TAP has been proposed to enhance antimicrobial efficacy and drug delivery. **Aim:** To evaluate and compare the antibacterial efficacy of calcium hydroxide, triple antibiotic paste, and nano-silica–incorporated triple antibiotic paste against *E. faecalis* biofilms using confocal laser scanning microscopy (CLSM). **Materials and Methods:** Dentin specimens prepared from extracted human teeth were sterilized and inoculated with *E. faecalis* to allow 7-day biofilm formation. The specimens were then treated with CH, TAP, or nano-silica TAP for one week. Bacterial viability was assessed using SYTO 9 and propidium iodide fluorescent staining and analyzed under CLSM. Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's post hoc test. **Results:** Nano-silica TAP demonstrated the highest antibacterial efficacy, with a significant reduction in *E. faecalis* biofilm viability compared with TAP and CH ($p < 0.05$). TAP alone showed moderate antibacterial activity, while calcium hydroxide exhibited the least effectiveness, with the highest percentage of viable bacterial cells. **Conclusion:** Nano-silica–incorporated triple antibiotic paste showed superior antimicrobial activity against *E. faecalis* biofilms compared to conventional TAP and calcium hydroxide. These findings indicate that nano-silica TAP is a promising intracanal medicament for enhanced endodontic disinfection; however, further *in-vivo* studies are required to validate its clinical applicability.

Key words: Biofilm, calcium hydroxide, endodontic disinfection, *Enterococcus faecalis*, nano-silica triple antibiotic paste, triple antibiotic paste

INTRODUCTION

Pulp necrosis resulting from trauma, anatomical abnormalities, or carious lesions in younger patients is commonly encountered in the field of endodontics. Since these teeth have not completed root formation, necrosis disrupts normal growth and compromises structural integrity.[1] Such teeth due to improper development have dentinal walls that are thin and more prone to fractures.

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This poses a challenge for long-term functional and restorative management. Additionally, open apex increases the risk of irrigant extrusion beyond the root canal system, leading to chemical irritation, potential flare-ups, and a compromised seal of the root canal filling.[2]

To achieve a successful outcome in endodontic regeneration protocols (ERP), maintaining aseptic conditions is crucial for sterilizing the lesion and promoting periradicular healing. ERPs aim to restore pulp vitality and promote periradicular healing in immature necrotic teeth.[3] Various techniques have been introduced to induce an artificial closure of the apex, a process known as apexification. Regenerative endodontics or apexification is often required to allow continued root development. Among these, mineral trioxide aggregate (MTA) or biodentine is commonly used in a single-visit procedure, promoting the formation of an apical plug that acts as a barrier. It is placed as a coronal barrier to seal the canal and support periapical healing. Alternatively, a multi-visit approach using calcium hydroxide (CH) is employed to stimulate the development of a hard tissue barrier at the root apex.[4,5]

Despite their effectiveness, both CH and MTA present clinical limitations. Prolonged exposure to CH weakens the root structure, increasing susceptibility to fractures, particularly during mastication.[6] On the other hand, MTA, when applied to teeth with large periapical lesions and funnel-shaped apices, may be overextended beyond the apex, complicating treatment.[7] To address these challenges, antibiotics have been introduced as intracanal medicaments to eradicate microbial biofilms and enhance the success of endodontic therapy. These medicaments work by reducing bacterial load, eliminating resistant species, and preventing reinfection. However, prolonged use of antibiotics may lead to resistance, dentin discoloration, and cytotoxic effects on periapical tissues. Therefore, controlled application and alternative antimicrobial strategies, such as CH and bioceramic materials, are often preferred for safer and effective root canal disinfection.[8]

Enterococcus faecalis is a resilient, facultative anaerobic bacterium commonly associated with persistent endodontic infections. It has the ability to form biofilms easily and withstand conventional antimicrobial treatments makes it a significant challenge in endodontic therapies. Increased resistance to various factors such as antibiotics and host immune with the presence of biofilm formation, often leading to treatment failure and reinfection.[9] Triple antibiotic paste (TAP), a combination medicament with the following: metronidazole, ciprofloxacin, and minocycline, introduced by Hoshino, has emerged as a potent intracanal medicament against *E. faecalis* biofilms.[10] The antibiotics present in the TAP have significant effects such as metronidazole, which interferes with anaerobic bacteria metabolism, while bacterial DNA replication is inhibited by ciprofloxacin, and synthesis of protein is interfered by minocycline. The combination of these antibiotics has a synergistic effect that eliminates bacteria and decreases the biofilm-associated resistance.[11]

TAP has been effective in several root canal therapies in recent years, especially in regenerative endodontic procedures and recurrent infections. It is effective in breaking down bacterial biofilms by deep penetration into the dentinal tubules which proves it a standard endodontic agent. However, literatures have shown limitations related to cytotoxicity and tooth discoloration with minocycline along with an increased risk of antibiotic resistance. These have paved the way to explore alternative strategies to optimize these limitations in endodontic applications.[12] The recommended concentration of TAP in regenerative procedures advised by the American Association of Endodontists is between 1 and 5 mg/mL as this concentration is deemed effective in sterilizing the root canal system. Higher concentrations apart from optimal cause stem cell toxicity, root structure weakening, and staining of the tooth.[13]

Innovative drug delivery methods have been experimented to overcome these challenges which will reduce the root canal disinfection by minimizing side effects. Local Drug Delivery Systems (LDDS) is an approach that allows direct application of these medicaments into the root canal. LDDS incorporates micro and nano-carriers such as microparticles, nanoparticles, micelles, and liposomes that will target infected areas, decrease systemic complications, and permit sustained drug release.[14,15] Endodontists have identified a new strategy that combines TAP with nano-silica, where the silica particles have a better penetration into the tubules, facilitating biofilm destruction and disruption of resistant bacteria. Furthermore, nano-silica possesses an additional antimicrobial property by producing reactive oxygen species that damages bacterial cell wall.[16]

Since the exact effects of nano-silica and TAP are not investigated, the study was directed to analyze and compare the antimicrobial effectiveness of CH, TAP, and nano-silica TAP against *E. faecalis* biofilms with the evaluation of bacterial viability post-treatment using confocal laser scanning microscopy (CLSM).

MATERIALS AND METHODS

The study has been approved by the scientific review board with the reference number SRB/SDC/PhD/ENDO-1901/24/348. The following intracanal medicaments were tested, and the corresponding methods used to evaluate their effectiveness are described below:

Intracanal medicaments

This study evaluated three different types of intracanal medicaments:

1. CH $Ca(OH)_2$ (Merck and Co., NJ) with saline: Prepared by mixing 250 mg of antibiotic (each) with 500 mg of $Ca(OH)_2$ in 1 mL of saline.
2. TAP: Containing 10 mg of all antibiotics in 10 mL of phosphate buffer solution (PBS)
3. Nano-silica TAP: 100 mg of silica nanoparticles and 100 mg of the respective antibiotic in 1 mL of distilled water
4. Control: Saline.

Antimicrobial testing and microscopic examination

Preparation of TAP and nanosilica TAP

TAP solution was prepared by adding an equal proportion of doxycycline, Flagyl, and ciprofloxacin of 300 mg of USP grade in distilled water of 3 mL. The mixtures were centrifuged at 3000 rpm for 15 min, and by using a sterile 25 mm syringe filter, the supernatant layers were filtered. A sterile conical flask was used for the synthesis. The solution consists of two solvents, ammonia and ethanol, with the concentrations 1.57 mL and 37 mL, respectively, to which water was added gently up to 5 mL. Upon stirring the mixture for an about 5 min, the addition of 3 mL of tetraethyl orthosilicate was done by stirring the mixture for an hour.

In nano-formulated TAP using nano silica as a carrier, the particle size typically below 100 nm was used which allows for deeper penetration into dentinal tubules and enhanced antimicrobial activity. The surface area of nano silica ranging from 500 to 1000 m^2/g, providing a high loading capacity for antibiotics was utilized. As for the concentration ratios of metronidazole, ciprofloxacin, and minocycline in nano TAP of 1:1:1 (mg/L) for doxycycline, Flagyl, and ciprofloxacin, respectively. The final concentration was adjusted to 100 mg of silica nanoparticles and 100 mg of the respective antibiotic in distilled water of 1 mL. Nano-silica-based antibiotic combinations were finally prepared by this procedure. Subsequently, the prepared combinations were dissolved in 1 mL of distilled water and subjected to mixing on a vortex mixer for a duration of 10–15 min. The resulting pellet was then dried using a hot air oven at a temperature of 60°C. Characterization of synthesized nanocarriers was carried out with X-ray diffraction, Fourier transform-infrared spectroscopy, and transmission electron microscopy. Drugs were loaded by dispersing 10 mg of the TAP drug in 10 mL of PBS and released with the solution pH 7.4 at 37°C.

Preparation of dentin samples

Dentin specimens were extracted from central incisors with fully developed roots. The teeth were positioned laterally to expose the flattest region of the root surface. A trephine drill was used, perpendicular to the dentinal walls (mesial and distal), to create dentin disks measuring 4 mm × 1.2 mm (diameter × thickness). To achieve a smooth pulp surface, the specimens were polished using 500- and 800-grit silicon carbide papers. During the process of preparation, a smear layer was formed which was eliminated by immersing the samples in 17% ethylenediaminetetraacetic acid for 5 min, followed by autoclave sterilization at 121°C for 20 min.

Cultivation of *E. faecalis* biofilm

All microbiological procedures were performed under aseptic conditions inside a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil). 15 µL standard strain *E. faecalis* (ATCC 4083) was grown overnight at 37°C in brain heart infusion (BHI) broth under aerobic conditions. The bacterial suspension was adjusted to a 1.0 optical density at 600 nm, corresponding to 10^9 colony-forming units/mL for *E. faecalis* (ATCC 4083) in a spectrophotometer (Ultraviolet-VISIBLI, Shimadzu, Japan) as per the 0.5 McFarland standard.

Infection of dentin specimens

The adjusted bacterial suspension was used to infect the dentin specimens. Each sample was placed in a 24-well multiwell plate, and 100 µL of *E. faecalis* culture mixed with 900 µL of BHI with a dentin block was added per well. The samples were incubated at 37°C for 21 days, with the BHI medium being refreshed every 48 h to support sustained biofilm growth.

Antimicrobial testing against *E. faecalis* biofilms

After the period of incubation, the biofilm-covered dentin samples were carefully washed to remove loosely attached planktonic bacteria with the help of 1 mL of distilled water. The specimens were then assigned randomly to four groups ($n = 20$):

- Group 1: CH
- Group 2: TAP
- Group 3: Nano-silica TAP
- Group 4: Control (Saline).

Contact test was carried out by immersing each dentin block in its respective intracanal medicament and incubated at 37°C for 7 days.

Microbiological analysis

The antimicrobial effect of the treatments was assessed using the SYTO 9/Propidium Iodide Live/Dead Staining Kit (Invitrogen, Eugene, OR).

- SYTO 9: A green fluorescent dye that stains both live and dead bacterial cells
- Propidium iodide: A red fluorescent nucleic acid stain that penetrates only bacterial membranes that are damaged, indicating dead cells.

Following treatment, the dentin blocks were rinsed with phosphate-buffered saline (PBS) and stained with 15 µL of the dye mixture in a dark environment for 15 min. The samples were then washed again before microscopic analysis. To quantify bacterial viability, BioImageL software (www.bioImageL.com) was used to measure the total biovolume of bacteria and the percentage of dead red-stained cells post-treatment.

Statistical analysis

The Shapiro-Wilks test was conducted to analyze the normality of the distribution. Non-parametric tests (Kruskal-Wallis and Dunn tests) were then carried out to analyze the variables.

RESULTS

The study highlights the effectiveness of various intracanal medicaments against *E. faecalis* biofilms. Table 1 presents the characteristics of CLSM including biovolume, biofilm thickness, and live/dead cell ratios among all groups. The highest percentage of viable cell was observed in the control and CH groups, indicating its lower antimicrobial efficacy. In contrast, both TAP and nano-silica TAP demonstrated significantly greater biofilm eradication, suggesting their superior antimicrobial potential.

Table 2 shows the mean, median, and SD of % of dead bacteria of all the groups on day 7 at both 500 m and 1000 m depths. Nano silica TAP shows the lowest mean value percentage of dead bacterial count which was statistically significant when compared to other groups at both 500 and 1000 m.

Figure 1 provides 3D micro-computed tomography reconstructions showing the distribution of intracanal medicaments in different sections of the root canal (coronal, middle, and apical thirds). The images illustrate how the medicaments interact with root canal structures, with Nano-silica TAP showing a more uniform distribution compared to CH and TAP. Figure 2 presents CLSM images, displaying the distribution of live and dead bacterial cells within biofilms post-treatment. In CH-treated biofilms (G1), a higher proportion of green-labelled live cells is evident, reinforcing its lower antimicrobial effect. In contrast, TAP (G2) and Nano-silica TAP (G3) groups exhibit predominantly red-stained dead cells, confirming their stronger bactericidal effects. Each image represents a 275 × 275 µm region.

Table 1: CLSM- biovolume, biofilm thickness, live/dead cell ratios after exposure to experimental medicaments for a week

Group	Biovolume[a] (µm ≥)	Biofilm thickness[b] (µm)	Live/dead ratio[c]
Calcium Hydroxide	520±30	20.5±1.2	0.80 (≈ 45% live cells) - A
TAP	45±8	1.8±0.4	0.0006 (≈ 0.06% live) - C
Nano Silica TAP	32±6	1.3±0.3	0.0003 (≈ 0.03% live) - C
Control	540±25	21.8±1.1	0.90 (≈ 47% live cells) -A

CLSM: Confocal laser scanning microscopy, TAP: Triple antibiotic paste. [a]Biovolume reflects the total 3D space occupied by the biofilm structure, [b]Thickness is the vertical height measured through Z-stacks in CLSM imaging. [c]Live/Dead Ratio is derived from SYTO 9/Propidium Iodide Live/Dead Staining Kit. Apical (A), middle (M), and coronal (C) thirds

Table 2: Mean, median, and SD of % of dead bacteria of all the groups on day 7 at both 500 m and 1000 m depths

Day-7	Groups	*n*	Mean	Median	Standard deviation	Standard error	95% Confidence interval for mean		Minimum	Maximum
							Lower bound	Upper bound		
500 m	Calcium hydroxide	5	54.53	53.250	19.1556	6.0575	40.834	68.241	25.60	87.83
	TAP	5	10.25	10.13	2.64251	0.4602	7.5622	11.4215	6.23	12.14
	Nano silica TAP	5	8.049	8.185	1.83616	0.5806	6.7355	9.3625	5.03	11.11
	Control	5	89.850	89.823	4.20028	1.3282	86.845	92.854	82.49	97.68
1000 m	Calcium Hydroxide	5	86.25	86.702	8.52145	2.6947	80.158	92.349	68.29	96.14
	TAP	5	11.49	11.23	2.63247	0.2412	8.4612	12.5214	7.02	13.25
	Nano Silica TAP	5	10.32	8.235	5.50710	1.7415	6.3855	14.264	5.28	21.63
	Control	5	99.28	99.435	0.36458	0.1152	99.020	99.541	98.69	99.69

SD: Standard deviation, TAP: Triple antibiotic paste

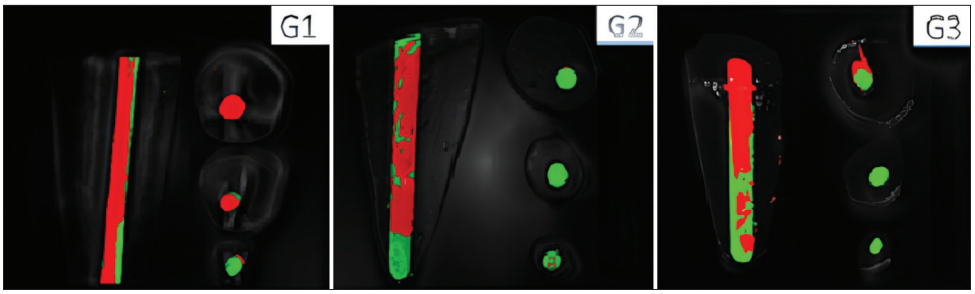

Figure 1: 3D micro-computed tomography reconstructions comparing initial (green) and final (red) images of intracanal dressings in root canals at coronal (C), middle (M), and apical (A) thirds. G1: Calcium hydroxide, G2: Triple antibiotic paste (TAP), G3: Nano-silica TAP

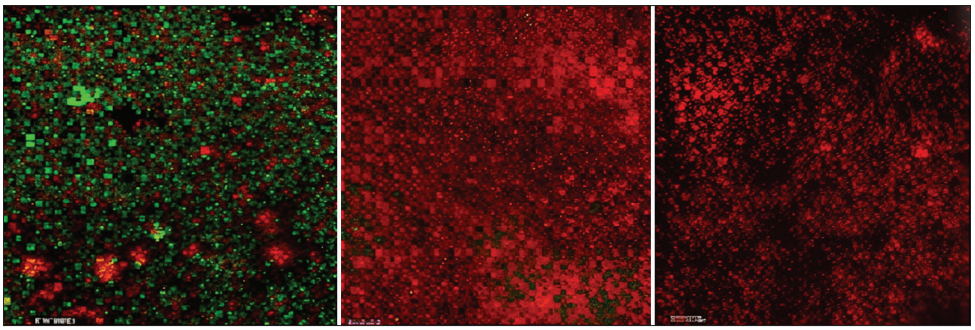

Figure 2: Confocal laser scanning microscopy images showing biofilms treated with calcium hydroxide, triple antibiotic paste (TAP), and nano-silica TAP. G1: Calcium hydroxide, G2: TAP, G3: Nano-silica TAP

DISCUSSION

This study compared the antimicrobial efficacy of CH, TAP, and Nano-silica TAP against *E. faecalis* biofilms. The results demonstrated that nano-silica TAP exhibited the highest antimicrobial activity, followed by TAP, while CH showed the least effectiveness in reducing biofilm viability. Given the fragile nature of dentin in immature teeth, revitalization therapy is considered the best approach for promoting continued root development in terms of both length and thickness. This technique involves inducing hemorrhage within a disinfected canal space, allowing the formation of a fibrin scaffold that supports new tissue growth. Proper antiseptic measures, including thorough irrigation without extrusion and minimal instrumentation, are crucial to preserving the dentin structure. Additionally, intracanal medication and coronal restoration are necessary to prevent bacterial reinfection.[17,18]

CH is extensively used as an intracanal medicament because of its high ~12.5 pH which creates an unfavorable environment for bacterial survival. Even though its effective against several endodontic pathogens, there is limited evidence on its effectiveness against *E. faecalis.* Adaptive mechanisms like efficient proton pumping help the organism to survive even in an alkaline pH, which reduces the bactericidal efficacy of CH. Literatures make it evident that CH effectively eliminates planktonic *E. faecalis*, but fails in its attempts to eliminate mature biofilms in deep dentinal tubules.[19] Regardless of this limitation, CH plays several vital roles such as the initiation process of hard tissue formation that helps in apexification procedures; enhancing the transforming growth factor -β1 growth factor release that supports stem cell migration, proliferation, and differentiation which is also important for maintaining the bacterial cell viability from the apical papilla over high-concentration antibiotic pastes,[20] all these makes it invaluable in apexification and regenerative endodontics. Even though CH is considered standard, it is not effective against the elimination of the biofilm created by the most persistent endodontic pathogen *E. faecalis*.[21] Similarly, our study also has proven that CH is the least effective than others with the presence of the highest percentage of live cells at the apical third of the root. This is due to the incomplete elimination of the bacterial biofilm structure by CH which makes it least effective than the TAP and nano-silica TAP which are antibiotic-based medicaments.

TAP, composed of metronidazole, ciprofloxacin, and minocycline, has been widely used as it is known for its antimicrobial activity that is broad-spectrum against both aerobic and anaerobic bacteria commonly found in infected root canals.[22] Metronidazole targets strict anaerobes, while ciprofloxacin is effective against gram-negative bacteria, and minocycline inhibits gram-positive bacteria. This combination allows TAP to penetrate deep into dentinal tubules, effectively eliminating persistent pathogens such as *E. faecalis* and *P. gingivalis*, which are commonly involved in endodontic infections.[23] The present study showed good efficacy against *E. faecalis* biofilms with less viable cells compared to CH but not less than nano-silica TAP. In the present study, TAP group showed live/dead cell ratio of 0.0006 in the coronal section. Similar studies have demonstrated that TAP significantly

reduces bacterial viability in biofilms, making it a highly effective intracanal medicament for regenerative endodontic procedures and root canal disinfection.[24] However, concerns regarding antibiotic resistance, potential cytotoxicity, and tooth discoloration necessitate have led to the exploration of alternative formulations, such as nano-silica TAP.[11]

The results showed that both TAP and nano-silica TAP had antibiofilm activity against *E. faecalis*. However, nano-silica TAP showed significantly the highest percentage of dead bacterial count at 500 m and 1000 m depth on both day 1 and day 7. Nano-silica TAP exhibited the highest antimicrobial activity in the coronal section similar to TAP but achieved a live/dead cell ratio of 0.0003, which is lower than that of the TAP group. This shows the potential of nano-silica-based formulations in optimizing antimicrobial effectiveness and regenerative outcomes in endodontic therapy. The high efficacy of nano-silica TAP compared to TAP and CH highlights its potential as an advanced intracanal medicament. Nano-silica TAP demonstrated superior antimicrobial activity, effectively eliminating *E. faecalis* biofilms more efficiently than conventional TAP. Hence, it can be concluded that Nano-silica TAP could be an effective intracanal medicament for root canal disinfection against *E. faecalis* biofilm under *in vitro* conditions.

The residual microbial antigens that are seen inside the root canal significantly influence the phenotypic behavior of transferred stem cells during regenerative procedures.[25] Consequently, persistent bacterial colonization poses a significant risk for periapical inflammation and intracanal reinfection, which may compromise treatment success. *E. faecalis* is notorious for forming resilient biofilms. Nano-silica–based formulations have shown potential in disrupting these biofilms more effectively than traditional pastes.[26] Similar results were evident with the incorporation of nano-silica, which enhances drug penetration into dentinal tubules, allowing for sustained antibacterial effects.[16] Similar to the present study, the combination of silica nanoparticles and antibiotics has exhibited concentration-dependent and time-dependent inhibitory effects on the growth of *E. faecalis* in a study that compared different concentrations of TAP, nano-silica (NP), and the combination of NP with TAP (NP-TAP).[27] In the present study, compared to nano-silica, TAP showed moderate efficacy while CH exhibited the least effectiveness, struggling to eradicate biofilm-forming bacteria due to the resistance mechanisms of *E. faecalis*. These findings suggest that Nano-silica TAP could serve as a more potent and reliable alternative for endodontic disinfection.

The study has a few limitations such as a short incubation period, limited specimens, lack of cytotoxicity, assessment, and variability in medicament penetration. A major limitation of this study is that this is an *in vitro* study conducted which analyzed the antimicrobial efficacy against a single microorganism, *E. faecalis*, whereas endodontic infections are polymicrobial. The stability of the materials on a long term and their effects on the physical properties of dentin were also not assessed. Hence, future research must focus on evaluating the efficacy against multiple organisms at various conditions along with the assessment of physical properties in the long term. Another limitation is that the antibiotic-based therapies often target drug diffusion, leading to reduced antimicrobial efficacy and potential toxicity to stem cells. To address this challenge, nanoparticle-based drug delivery systems have been introduced, incorporating materials such as nano-silica to improve drug retention and efficacy.[28] Nano-silica serves as a dental filler, enhancing mechanical properties and controlling drug release. When incorporated into TAP, nano-silica significantly improves the therapeutic index and pharmacokinetics, thereby enhancing dentin-pulp complex regeneration and infection control.

CONCLUSION

The study confirms the superior antimicrobial efficacy of nano-silica TAP, followed by TAP compared to CH in eliminating *E. faecalis* biofilms. The findings suggest that incorporating nano-silica enhances drug penetration into dentinal tubules, ensuring sustained antibacterial effects. TAP demonstrated moderate efficacy, while CH exhibited the least effectiveness, struggling to eradicate mature biofilms. Despite its advantages, nano-silica TAP requires further validation through long-term *in vivo* studies to evaluate its potential cytotoxic effects and clinical performance. The study highlights the importance of advanced drug delivery systems in improving endodontic disinfection outcomes and highlights nano-silica TAP as a promising alternative to conventional intracanal medicaments.

DATA AVAILABILITY STATEMENT

Data are available in the article; further details can be requested by mail to the corresponding author.

REFERENCES

1. Asgary S, Shamszadeh S, Nosrat A, Aminoshariae A, Sabeti M. Management strategies for immature teeth with pulp necrosis: An umbrella review of systematic reviews. Iran Endod J 2024;19:242-53.
2. Yassen GH, Chu TM, Eckert G, Platt JA. Effect of medicaments used in endodontic regeneration technique on the chemical structure of human immature radicular dentin: An *in vitro* study. J Endod 2013;39:269-73.
3. Pulyodan MK, Paramel Mohan S, Valsan D, Divakar N, Moyin S, Thayyil S. Regenerative endodontics: A paradigm shift in clinical endodontics. J Pharm Bioallied Sci 2020;12:S20-6.

4. Demiriz L, Hazar Bodrumlu E. Retrospective evaluation of healing of periapical lesions after unintentional extrusion of mineral trioxide aggregate. J Appl Biomater Funct Mater 2017;15:e382-6.
5. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: Biological basis of regenerative endodontic procedures. Pediatr Dent 2013;35:129-40.
6. Nazzal H, Duggal MS. Regenerative endodontics: A true paradigm shift or a bandwagon about to be derailed? Eur Arch Paediatr Dent 2017;18:3-15.
7. Torabinejad M, Parirokh M, Dummer PM. Mineral trioxide aggregate and other bioactive endodontic cements: An updated overview - part II: Other clinical applications and complications. Int Endod J 2018;51:284-317.
8. Madla-Cruz E, Villanueva-Pérez VD, De la Garza-Ramos MA, Flores-Treviño JJ, Rodríguez-Delgado I, López-Martinez F. An *in vitro* evaluation of endodontic sealers and an antibiotic to assess their antimicrobial effect against *Enterococcus faecalis*. Biomater Investig Dent 2024;11:40646.
9. Yang S, Meng X, Zhen Y, Baima Q, Wang Y, Jiang X, *et al.* Strategies and mechanisms targeting *Enterococcus faecalis* biofilms associated with endodontic infections: A comprehensive review. Front Cell Infect Microbiol 2024;14:1433313.
10. Taneja S, Kumari M. Use of triple antibiotic paste in the treatment of large periradicular lesions. J Investig Clin Dent 2012;3:72-6.
11. Parhizkar A, Nojehdehian H, Asgary S. Triple antibiotic paste: Momentous roles and applications in endodontics: A review. Restor Dent Endod 2018;43:e28.
12. Malu K, Khubchandani M. Triple antibiotic paste: A suitable medicament for intracanal disinfection. Cureus 2022;14:e29186.
13. Bains R, Tikku AP, Verma P, Pandey P. Antimicrobial efficacy of triple antibiotic paste in teeth with primary endodontic infection: A systematic review. Asian J Oral Health Allied Sci 2021;11:1-7.
14. Wei Y, Deng Y, Ma S, Ran M, Jia Y, Meng J, *et al.* Local drug delivery systems as therapeutic strategies against periodontitis: A systematic review. J Control Release 2021;333:269-82.
15. Makvandi P, Josic U, Delfi M, Pinelli F, Jahed V, Kaya E, *et al.* Drug delivery (nano)platforms for oral and dental applications: Tissue regeneration, infection control, and cancer management. Adv Sci (Weinh) 2021;8:2004014.
16. Hameed S, Antony DP, Shanmugam R, Raghu S, Adimulapu HS. Enhancing antimicrobial efficacy and synergistic effects of nano-silica-based combinations with doxycycline, metronidazole, and ciprofloxacin against *Enterococcus faecalis* biofilms. Cureus 2024;16:e54668.
17. Lin LM, Ricucci D, Huang GT. Regeneration of the dentine-pulp complex with revitalization/ revascularization therapy: Challenges and hopes. Int Endod J 2014;47:713-24.
18. Karobari MI, Adil AH, Assiry AA, Basheer SN, Noorani TY, Pawar AM, *et al.* Herbal medications in endodontics and its application-a review of literature. Materials (Basel) 2022;15:3111.
19. Teja KV, Janani K, Srivastava KC, Shrivastava D, Natoli V, Di Blasio M, *et al.* Comparative evaluation of antimicrobial efficacy of different combinations of calcium hydroxide against *Enterococcus faecalis*. BMC Oral Health 2023;23:849.
20. Liu C, Li Y, Li Y, Li Z, Han G. Synergistic antibacterial effect of ginsenoside Rh2 and calcium hydroxide on *Enterococcus faecalis*. Odontology 2025;113:111-25.
21. Chandwani ND, Maurya N, Nikhade P, Chandwani J. Comparative evaluation of antimicrobial efficacy of calcium hydroxide, triple antibiotic paste and bromelain against *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent Endod 2022;25:63-7.
22. Kumari A, Sawhney H, Kashwani R, Gupta G, Das SJ. Triple antibiotics: A synergistic approach to combating infection. IP Indian J Conserv Endod 2023;8:189-92.
23. Raut AW, Shenoi PR. A comparative analysis of antibacterial efficacy of triple antibiotic paste and modified triple antibiotic paste against *Enterococcus faecalis*: An *in vitro* study. Bharati Vidyapeeth J Dent Allied Sci 2024;1:8-11.
24. Nasim I, Kanth Jaju K, Shamly M, Vishnupriya V, Jabin Z. Effect of nanoparticle based intra-canal medicaments on root dentin micro-hardness. Bioinformation 2022;18:226-30.
25. Sabrah AH, Yassen GH, Spolnik KJ, Hara AT, Platt JA, Gregory RL. Evaluation of residual antibacterial effect of human radicular dentin treated with triple and double antibiotic pastes. J Endod 2015;41:1081-4.
26. Verma P, Nosrat A, Kim JR, Price JB, Wang P, Bair E, *et al.* Effect of residual bacteria on the outcome of pulp regeneration *in vivo*. J Dent Res 2017;96:100-6.
27. Hameed S, Antony DP, Shanmugam R. Assessing the antimicrobial efficacy of triple antibiotic paste (TAP) and nano-silica-enhanced TAP (TAP-N) against *Enterococcus faecalis*: A time kill curve assay study. Afr J Biol Sci 2024;6:1516-24.
28. Parhizkar A, Asgary S. Local drug delivery systems for vital pulp therapy: A new hope. Int J Biomater 2021;2021:5584268.

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