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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

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ABSTRACT:

Introduction:
This study aimed to evaluate the antimicrobial efficacy of a combination of three antibiotics, TAP (doxycycline, Flagyl, and ciprofloxacin), against the pathogenic bacterium E. faecalis, which is known to cause various human infections. The assessment was conducted through a time kill curve assay, a method that assesses the bactericidal or bacteriostatic effects of antimicrobial agents across different concentrations and time intervals. Additionally, the study incorporated the use of silica nanoparticles (nano-silica) as carriers for the antibiotics, with the hypothesis that this combination would enhance their antimicrobial effectiveness.

Methods:
The synthesis of nano-silica involved a gentle combination of ammonia, ethanol, water, and tetraethyl orthosilicate (TEOS) in a sterile conical flask. Subsequent centrifugation and drying were carried out to prepare the nano-silica. Silica nanoparticles were used as carriers for the Triple Antibiotics Paste.The time kill curve assay was performed by inoculating sterilized Muller Hinton broth with an overnight E. faecalis bacterial suspension, ensuring a bacterial concentration of approximately 5×10^5 CFU/mL. Test tubes were treated with different concentrations of TAP, nano-silica (NP), and the combination of NP with TAP (NP-TAP). The antimicrobial effects were evaluated at various time intervals (1hr, 2hr, 3hr, 4hr, and 5hr) by measuring the optical density at a wavelength of 600 nm using a spectrophotometer. A control group without treatment served as a baseline for comparison.

Results:
The time kill curve assay results revealed concentration-dependent and time-dependent inhibitory effects of TAP, NP, and NP-TAP on the growth of E. faecalis. As the concentration of TAP, NP, and NP-TAP increased, there was a more pronounced inhibition of microbial growth. Extended incubation time also enhanced the inhibitory effect of these antimicrobial agents, highlighting the time-dependent nature of their action.

Comparing the three groups, NP-TAP exhibited enhanced antimicrobial activity against E. faecalis, with lower bacterial growth values observed at equivalent concentrations and time points. This suggests that the inclusion of silica nanoparticles may improve the delivery and effectiveness of TAP in combating bacterial infections.The control group consistently displayed higher microbial growth, underscoring the significance of TAP, NP, and NP-TAP in inhibiting bacterial proliferation.

Conclusion:
This study demonstrated that TAP, NP, and NP-TAP exhibit concentration-dependent and time-dependent inhibitory effects on the growth of E. faecalis. NP-TAP, the combination of silica nanoparticles and antibiotics, showed the most potent antimicrobial activity. These findings suggest the potential use of TAP and NP-TAP as effective agents for controlling E. faecalis infections. Further research is needed to explore their clinical applications and safety profiles. The use of nano-silica as a carrier for antibiotics offers a promising avenue for enhancing the effectiveness of antimicrobial treatments.

Keywords: E. Faecalis, Nanoparticle, Silica Nanoparticles, Triple Antibiotics Paste.

1. INTRODUCTION

Enterococcus faecalis is a Gram-positive bacterium frequently associated with persistent and refractory infections in root canals following unsuccessful endodontic treatments. The strains isolated from these cases exhibit a range of virulence factors contributing to their pathogenic potential. Notably, the presence of virulence genes, such as efaA, esp, ace, cylA, gelE, and asa, has been detected in varying proportions among different strains [1] [2] [3]. Furthermore, some strains display the ability to form biofilms, indicating their capacity to adhere and persist within the root canal environment [4] [5]. Additionally, certain strains exhibit gelatinase production and β-lactamase resistance, suggesting potential involvement in tissue degradation and antibiotic resistance. The high genomic diversity observed among these strains underscores the existence of multiple clonal types [6].

Understanding the virulence factors of Enterococcus faecalis is crucial for devising effective treatment strategies to address its pathogenic potential in endodontic treatment failures. Notably, nanotechnology, particularly the use of nanoparticles, has emerged as a promising avenue in endodontics to enhance antimicrobial efficacy. In vitro studies have extensively investigated various types of nanoparticles for their antimicrobial properties in root canal infections. Silver nanoparticles, in particular, have demonstrated sustained antimicrobial activity against root canal infections [7] [8]. Additionally, nanoparticles such as glass bioactive nanoparticles, calcium derivative-based nanoparticles, and bioactive non-organic nanoparticles have exhibited superior antimicrobial activity compared to conventional antimicrobial procedures [9].

Despite promising results in vitro, there remains a need for further investigation into the optimal particle size and duration of contact for achieving effective antimicrobial action.
In nanoparticle research, mesoporous silica nanoparticles (MSNs) have garnered attention for their potential to infiltrate dentinal tubules effectively. Previous studies have explored the use of fluorescence-labeled MSNs to investigate their infiltration profiles into dentin, demonstrating their ability to accumulate on root canal walls and penetrate dentinal tubules [10]. Furthermore, MSNs have been employed for tubular occlusion to treat dentin hypersensitivity, showcasing significant differences in tubular occlusion compared to conventional desensitizers [11], [12].

The present study aims to contribute to this body of knowledge by assessing the antimicrobial efficacy of Triple Antibiotic Paste (TAP) and Nano silica-Enhanced TAP (TAP-N) against Enterococcus faecalis. Utilizing a time kill curve Assay, this study aim to elucidate the temporal dynamics of bacterial growth inhibition, providing valuable insights into the potential enhanced efficacy of TAP-N over conventional TAP in combating Enterococcus faecalis in endodontic applications.

2. MATERIALS AND METHODS

Synthesis of Nano Silica
In a sterile conical flask, a combination of 1.57 mL of ammonia (as the solvent), 37 mL of ethanol (also a solvent), and 5 mL of water was gently combined. After stirring the mixture for 5 minutes, an additional 3 mL of TEOS was introduced and the stirring process continued for an hour. Subsequently, the silica nanoparticles were separated by subjecting the mixture to centrifugation at 10,000 rpm for 30 minutes. The resulting pellet was then dried using a hot air oven at a temperature of 60°C.

Preparation of Silica Nanoparticles Based Antibiotic Combinations:
Silica nanoparticles were combined with antibiotics, including doxycycline, Flagyl, and ciprofloxacin, in a 1:1 ratio. Each combination consisted of 100 mg of silica nanoparticles and 100 mg of the respective antibiotic. Subsequently, these combinations were dissolved in 1 mL of distilled water and subjected to mixing on a vortex mixer for a duration of 10-15 minutes. This procedure was conducted to prepare the nano-silica-based antibiotic combinations.

Time Kill Curve Assay
To perform time kill curve assay, sterilized Muller Hinton broth is prepared and dispensed into five test tubes. These tubes are then inoculated with an overnight E. faecalis bacterial suspension, ensuring a bacterial concentration of approximately 5×10^5 CFU/mL. Subsequently, three of the test tubes are treated with different concentrations of TAP and nanosilica-based TAP (25 µL, 50 µL, and 100 µL), while the fourth tube serves as a standard amoxyrite, and the fifth tube includes an aerobic bacterial suspension as an positive control. The test tubes are incubated under aerobic conditions at 37°C for varying time intervals (1hr, 2hr, 3hr, 4hr, and 5hr). At regular intervals, the percentage of dead bacterial cells is assessed by measuring the optical density at a wavelength of 600 nm using a spectrophotometer. (Figure 1)

3. RESULT AND DISCUSSION

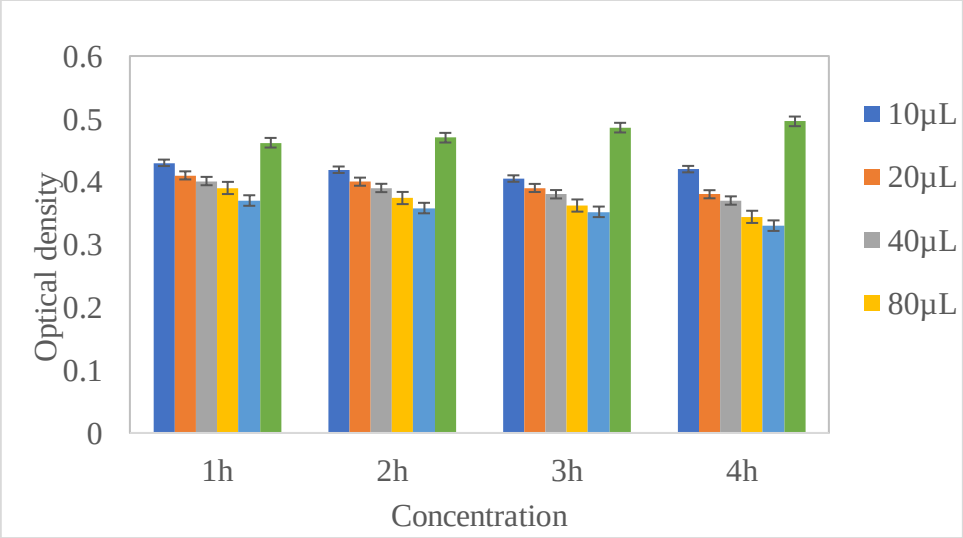

Figure 1: Time kill curve assay of NP-TAP against E.faecalis

In the Minimum Inhibitory Concentration (MIC) assay of NP-TAP against E.faecalis, a comprehensive assessment revealed the impact of NP-TAP at various concentrations (ranging from 10µL to 160µL) and time points (1h, 2h, 3h, and 4h). The results demonstrate a clear dose-dependent and time-dependent inhibitory effect on the growth of E.faecalis. As the concentration of NP-TAP increased, a greater inhibition of microbial growth was observed, indicating the compound's potency. Additionally, as the incubation time extended, the inhibitory effect became more pronounced. In contrast, the control group exhibited higher microbial growth without NP-TAP treatment, providing a baseline for comparison. These findings underscore the potential of NP-TAP as a promising antimicrobial agent against E.faecalis, offering valuable insights into its effectiveness in controlling bacterial proliferation under varying conditions. (Figure 2)

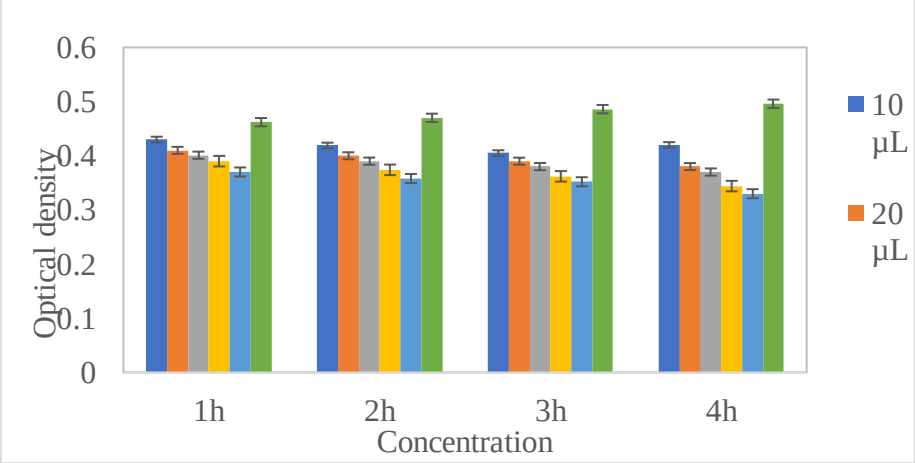

Figure 2: Time kill curve assay of silica nanoparticle against E.faecalis

The MIC assay of silica nanoparticles against E.faecalis revealed concentration-dependent inhibition of bacterial growth. At a concentration of 10µL, E.faecalis exhibited a minimal inhibitory effect, with growth values ranging from 0.43 at 1 hour to 0.42 at 4 hours. As the concentration of silica nanoparticles increased to 20µL, 40µL, and 80µL, a progressively more substantial growth inhibition was observed at all time points, with values decreasing over time. Notably, at 160µL, the highest concentration tested, the inhibitory effect was most prominent, resulting in growth values as low as 0.336 at 4 hours. These findings emphasize the strong concentration-dependent antimicrobial activity of silica nanoparticles against E.faecalis, indicating their potential as an effective agent for controlling bacterial proliferation, especially at higher concentrations and extended incubation times.

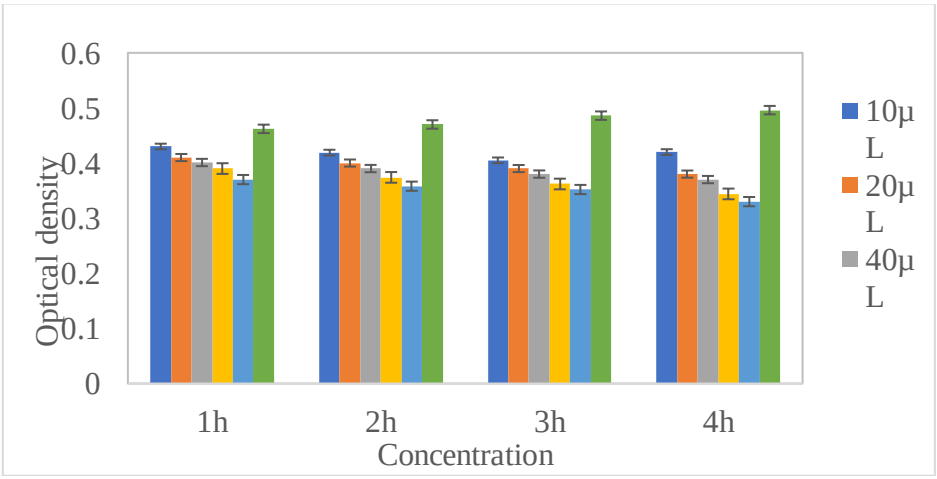

Figure 3: Time kill curve assay of TAP against E.faecalis

In figure 3 the time kill curve assay of TAP against E.faecalis, the data illustrates the concentration and time-dependent impact on E.faecalis growth. At the lowest concentration of 10µL, TAP resulted in minimal inhibitory effects, with bacterial growth values ranging from 0.43 at 1 hour to 0.42 at 4 hours. As the TAP concentration increased to 20µL, 40µL, and 80µL, a progressively more substantial growth inhibition was observed across all time points, with values consistently decreasing over time. Notably, at the highest concentration tested, 160µL, TAP exhibited the most pronounced inhibitory effect, resulting in growth values as low as 0.33 at 4 hours. These findings highlight the strong concentration-dependent antimicrobial activity of TAP against E.faecalis, suggesting its potential as an effective agent for controlling bacterial proliferation, especially at higher concentrations and with extended incubation times. And the

control group, which did not receive TAP treatment, displayed higher microbial growth, providing a baseline for comparison.

4. DISCUSSION

The present study aimed to assess the antimicrobial efficacy of TAP (doxycycline, Flagyl, and ciprofloxacin) against E. faecalis, a pathogenic bacterium known to cause various infections in humans. This assessment was conducted using a time kill curve assay, a common method for evaluating the bactericidal or bacteriostatic effects of antimicrobial agents over a range of concentrations and time intervals.

Various in-vitro studies have investigated the antimicrobial effectiveness of different materials against Enterococcus faecalis. In one study, a newly developed 3C antibiotic paste (comprising ciprofloxacin, clindamycin, and cefaclor) exhibited superior antimicrobial efficacy compared to the conventional Triple Antibiotic Paste (TAP) [13]. Another investigation demonstrated TAP against chitosan, calcium hydroxide, and normal saline, revealing that TAP with chitosan demonstrated the highest antimicrobial efficacy [14]. Evaluating calcium hydroxide paste with PLUS points as an endodontic dressing, another study concluded that the paste was more proficient in eliminating E. faecalis growth within infected canals [15]. Furthermore, a comparison of nano chitosan and chlorhexidine with sodium hypochlorite found no statistically significant differences among the tested groups [16]. Lastly, an examination of chlorhexidine, nano-chitosan, and their combination indicated that chlorhexidine with ultrasonic activation exhibited the most potent antimicrobial effects against E. faecalis [17].

Several in vitro studies assessed the antimicrobial effectiveness of various intracanal medicaments against common endodontic pathogens. One investigation, focusing on chlorhexidine, calcium hydroxide, and cetylpyridinium chloride, revealed that cetylpyridinium chloride demonstrated the most significant reduction in colony-forming units (CFU) values and, therefore, was the most efficacious medicament [18]. Another study explored the antibacterial efficacy of the probiotics BIFILAC and VSL 3 against Enterococcus faecalis, finding both probiotics effective in decreasing CFU counts [19]. In another previous research work, the antimicrobial effectiveness of herbal agents such as curcumin, propolis, and aloe vera, with or without a carrier, was compared against E. faecalis, with triple antibiotic paste emerging as the most effective medicament [20]. Lastly, a study examining five endodontic sealers against E. faecalis, Fusobacterium nucleatum, and Porphyromonas gingivalis identified Endomethasone and AH Plus as having notable antimicrobial effects [21].

The synthesis of silica nanoparticles (nano-silica) is a crucial aspect of this study, as these nanoparticles serve as carriers for the antibiotics and can potentially enhance their effectiveness. The synthesis process involved the gentle combination of ammonia, ethanol, water, and tetraethyl orthosilicate (TEOS), followed by centrifugation and drying. The successful preparation of nano-silica is essential, as any impurities or inconsistencies in the nanoparticles' characteristics could affect the results of the time kill curve assay.

The time kill curve assay results clearly demonstrate the antimicrobial activity of TAP, NP and NP-TAP against E. faecalis. The study reveals a concentration-dependent and time-dependent inhibitory effect on the growth of E. faecalis for both TAP and NP-TAP. As the concentration of TAP or TAP-N increased, a greater inhibition of microbial growth was observed, suggesting the potency of these compounds. Additionally, as the incubation time extended, the inhibitory effect became more pronounced, indicating the time-dependent aspect of their antimicrobial action.

Comparing TAP, NP and NP-TAP, the data shows that the combination of TAP with silica nanoparticles enhances the antimicrobial activity against E. faecalis. This is evident in the lower bacterial growth values observed for TAP-N at equivalent concentrations and time points. The findings suggest that the inclusion of silica nanoparticles may improve the delivery and effectiveness of TAP against E. faecalis, making it a more promising antimicrobial agent for combating bacterial infections.Furthermore, the study includes a control group that did not receive TAP, NP and NP-TAP treatment, serving as a baseline for comparison. The control group consistently exhibited higher microbial growth, highlighting the importance of the antimicrobial agents in inhibiting bacterial proliferation. These results reinforce the significance of TAP and TAP-N as effective agents for controlling E. faecalis growth.

The concentration-dependent effects observed in the study emphasize the importance of optimizing the dosage of TAP, NP and NP-TAP for specific clinical applications. Higher concentrations of both TAP and NP-TAP showed more substantial inhibitory effects, suggesting that a higher dosage might be necessary for severe infections or for cases with a higher initial bacterial load.
Overall, this study provides valuable insights into the antimicrobial efficacy of TAP, NP and NP-TAP against E. faecalis through a time kill curve assay. The data indicates that all samples exhibit concentration-dependent and time-dependent inhibitory effects on E. faecalis growth, with NP-TAP showing enhanced antimicrobial activity. These findings support the potential use of TAP and NP-TAP as effective agents for controlling E. faecalis infections, and further research is needed to explore their clinical applications and safety profiles.

5. CONCLUSION

This current study underscores the potential of TAP (doxycycline, Flagyl, and ciprofloxacin) silica nanoparticles and the novel combination of TAP with silica nanoparticles (NP-TAP) as potent antimicrobial agents against E. faecalis. Through a comprehensive time kill curve assay, we observed clear evidence of concentration-dependent and time-dependent inhibitory effects on E. faecalis growth. The inclusion of silica nanoparticles in the NP-TAP combination significantly enhanced the antimicrobial activity compared to TAP alone. These findings offer a promising avenue for improving the efficacy of antimicrobial treatments against E. faecalis infections. The control group's consistently higher microbial growth further underscores the importance of TAP and NP-TAP in inhibiting bacterial proliferation, emphasizing their potential in clinical applications. As the concentration-dependent effects were evident, optimizing the dosage of TAP and NP-TAP for specific clinical scenarios becomes crucial. Higher concentrations of these agents demonstrated more substantial inhibitory effects, suggesting their potential utility in cases with a higher initial bacterial load or severe infections. This study contributes valuable insights into the development of effective strategies for combating E. faecalis infections, offering potential clinical applications for TAP and NP-TAP. However, further research is needed to explore their safety profiles and clinical potential, paving the way for innovative approaches to address bacterial infections and enhance patient care. The use of nano-silica as a carrier for antibiotics opens new doors for improving the delivery and effectiveness of antimicrobial treatments in the fight against pathogenic bacteria.

6. REFERENCES

1. P. A. Francisco, P. I. da G. Fagundes, J. C. Lemes-Junior, A. R. Lima, M. R. Z. Passini, and B. P. F. A. Gomes, “Pathogenic potential of Enterococcus faecalis strains isolated from root canals after unsuccessful endodontic treatment,” Clin. Oral Investig., vol. 25,

no. 9, pp. 5171–5179, Sep. 2021, doi: 10.1007/s00784-021-03823-w. Available: http://dx.doi.org/10.1007/s00784-021-03823-w

2. G. O. Zoletti, E. M. Pereira, R. P. Schuenck, L. M. Teixeira, J. F. Siqueira Jr, and K. R. N. dos Santos, "Characterization of virulence factors and clonal diversity of Enterococcus faecalis isolates from treated dental root canals," Res. Microbiol., vol. 162, no. 2, pp. 151–158, Feb. 2011, doi: 10.1016/j.resmic.2010.09.018. Available: http://dx.doi.org/10.1016/j.resmic.2010.09.018
3. F. Saffari, M. H. Sobhanipoor, A. Shahravan, and R. Ahmadrajabi, "Virulence genes, antibiotic resistance and capsule locus polymorphisms inEnterococcus faecalisisolated from canals of root-filled teeth with periapical lesions," Infect. Chemother., vol. 50, no. 4, p. 340, 2018, doi: 10.3947/ic.2018.50.4.340. Available: http://dx.doi.org/10.3947/ic.2018.50.4.340
4. X. Zhu, Q. Wang, C. Zhang, G. S. P. Cheung, and Y. Shen, "Prevalence, phenotype, and genotype of Enterococcus faecalis isolated from saliva and root canals in patients with persistent apical periodontitis," J. Endod., vol. 36, no. 12, pp. 1950–1955, Dec. 2010, doi: 10.1016/j.joen.2010.08.053. Available: http://dx.doi.org/10.1016/j.joen.2010.08.053
5. I. Portenier, T. M. T. Waltimo, and M. Haapasalo, "Enterococcus faecalis– the root canal survivor and 'star' in post-treatment disease," Endod. Topics, vol. 6, no. 1, pp. 135–159, Nov. 2003, doi: 10.1111/j.1601-1546.2003.00040.x. Available: http://dx.doi.org/10.1111/j.1601-1546.2003.00040.x
6. M. Samiei, A. Farjami, S. M. Dizaj, and F. Lotfipour, "Nanoparticles for antimicrobial purposes in Endodontics: A systematic review of in vitro studies," Mater. Sci. Eng. C Mater. Biol. Appl., vol. 58, pp. 1269–1278, Jan. 2016, doi: 10.1016/j.msec.2015.08.070. Available: http://dx.doi.org/10.1016/j.msec.2015.08.070
7. P. G. Chandak et al., "Nanoparticles in Endodontics - A Review," J. Evol. Med. Dent. Sci., vol. 10, no. 13, pp. 976–982, Mar. 2021, doi: 10.14260/jemds/2021/209. Available: http://dx.doi.org/10.14260/jemds/2021/209
8. S. Bhandi et al., "Antimicrobial efficacy of silver nanoparticles as root canal irrigant's: A systematic review," J. Clin. Med., vol. 10, no. 6, p. 1152, Mar. 2021, doi: 10.3390/jcm10061152. Available: http://dx.doi.org/10.3390/jcm10061152
9. F. Hayat et al., "Nanoparticles in endodontics," in Biomaterials in Endodontics, Elsevier, 2022, pp. 195–209. doi: 10.1016/b978-0-12-821746-7.00004-8. Available: http://dx.doi.org/10.1016/b978-0-12-821746-7.00004-8
10. X. Li, X. Li, S. Wang, K. C.-F. Leung, C. Zhang, and L. Jin, "Infiltration and profiles of mesoporous silica nanoparticles in dentinal tubules," ACS Biomater. Sci. Eng., vol. 4, no. 4, pp. 1428–1436, Apr. 2018, doi: 10.1021/acsbiomaterials.7b00919. Available: http://dx.doi.org/10.1021/acsbiomaterials.7b00919
11. L. Tian et al., "Effect of mesoporous silica nanoparticles on dentinal tubule occlusion: An in vitro study using SEM and image analysis," Dent. Mater. J., vol. 33, no. 1, pp. 125–132, 2014, doi: 10.4012/dmj.2013-215. Available: http://dx.doi.org/10.4012/dmj.2013-215
12. L. Zhang, H. Sun, J. Yu, H. Yang, F. Song, and C. Huang, "Application of electrophoretic deposition to occlude dentinal tubules in vitro," J. Dent., vol. 71, pp. 43–48, Apr. 2018, doi: 10.1016/j.jdent.2018.01.012. Available: http://dx.doi.org/10.1016/j.jdent.2018.01.012
13. A. Chandra and N. Thosar, "Comparative evaluation of antimicrobial efficacy of 3C antibiotic paste with triple antibiotic paste as root canal filling material for primary teeth: An in-vitro study," F1000Res., vol. 12, p. 754, Jun. 2023, doi: 10.12688/f1000research.133458.1. Available: http://dx.doi.org/10.12688/f1000research.133458.1

14. T. Ul Nisa, I. Gulzar, and N. Gupta, "In-vitro evaluation of antimicrobial efficacy of triple antibiotic paste with chitosan against Enterococcus faecalis," Int J Sci Healthcare Res, vol. 8, no. 1, pp. 240–245, Mar. 2023, doi: 10.52403/ijshr.20230134. Available: http://dx.doi.org/10.52403/ijshr.20230134
15. P. G. Rumhein, K. J. Layous, H. Achour, M. M. Mousa, H. Deeb, and M. Y. Hajeer, "Antimicrobial efficacy of two different calcium hydroxide endodontic dressings on the eradication of Enterococcus faecalis in single-rooted canals: An in vitro study," Cureus, Jun. 2023, doi: 10.7759/cureus.40056. Available: http://dx.doi.org/10.7759/cureus.40056
16. S. Mohamed Nour ElDeen, W. Kamel, M. Rokaya, and M. Aly Sherif, "Evaluation of antimicrobial efficacy of nano chitosan and chlorohexidine versus sodium hypochlorite as final rinse against Enterococcus faecalis," Al-Azhar Journal of Dentistry, vol. 10, no. 1, Jan. 2023, doi: 10.58675/2974-4164.1460. Available: http://dx.doi.org/10.58675/2974-4164.1460
17. H. Sadek, M. Hassan, and H. Elshishtawy, "Antibacterial effect of chlorhexidine, nano-chitosan against entercoccus faecalis with and without using ultrasonic activation. (An in vitro study)," Egypt. Dent. J., vol. 68, no. 4, pp. 3915–3923, Oct. 2022, doi: 10.21608/edj.2022.136335.2096. Available: http://dx.doi.org/10.21608/edj.2022.136335.2096
18. I. Sangita, S. Vishwanath, K. Sadasiva, A. Ramachandran, Y. Thanikachalam, and V. Ramya, "Influence of simulated wound exudate on the antimicrobial efficacy of various intracanal medicaments against Enterococcus faecalis: An in vitro study," Cureus, May 2023, doi: 10.7759/cureus.38677. Available: http://dx.doi.org/10.7759/cureus.38677
19. B. N. Reddy, S. Sankar, S. Ravi, H. B. Ananthan, S. Sankar, and S. N. Natarajan, "Evaluation of antibacterial efficacy of two commercially available probiotics as intracanal medicament against Enterococcus faecalis: An in vitro study," J. Contemp. Dent. Pract., vol. 24, no. 3, pp. 157–161, May 2023, doi: 10.5005/jp-journals-10024-3466. Available: http://dx.doi.org/10.5005/jp-journals-10024-3466
20. G. Patri, K. Sheetal, P. Pradhan, P. Agrawal, and S. Lata, "Comparative evaluation of the antibacterial efficacy of herbal agents as intracanal medicaments individually or in combination with chitosan: An in vitro RTPCR study," J. Int. Oral Health, vol. 15, no. 1, p. 89, 2023, doi: 10.4103/jioh.jioh_154_22. Available: http://dx.doi.org/10.4103/jioh.jioh_154_22
21. G. El and H. Mai, Comparative evaluation of bromelain, double antibiotic paste, and calcium hydroxide as intracanal medicaments against Enterococcus faecalis: an in-vitro study. 2023. doi: 10.4103/tdj.tdj_57_22. Available: http://dx.doi.org/10.4103/tdj.tdj_57_22