EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 3423:3431, OCTOBER, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 25-05-2025 • Accept Date : 06-07-2025 • Available online: 10-10-2025 • DOI : 10.21608/edj.2025.388748.3497

EVALUATION OF ANTIMICROBIAL EFFECT OF MAGNESIUM OXIDE NANO PARTICLES AND CONVENTIONAL CALCIUM HYDROXIDE AS INTRACANAL MEDICATION AGAINST ENTEROCOCCUS FAECALIS (AN IN-VITRO STUDY)

Remon Girgis Yacoub*, Rehab M Abd El-Baky**, Essam Fawzy Mahmoud*** *and* Mohamed A. Mawhoup****

ABSTRACT

Aim: evaluate the antibacterial effect of nano based intracanal medicaments Magnesium Oxide Nanoparticles (nMgO) comparing to conventional Calcium hydroxide.

Materials and methods: 80 human uni-radicular teeth were decoronated then instrumented using Protaper universal Rotary File System till file (F4). After being infected with E. faecalis the samples were allocated into four equal experimental groups based on the type of intracanal medicament used namely: Magnesium Oxide Nanoparticles paste, Calcium hydroxide paste, Magnesium Oxide Nanoparticles plus Calcium hydroxide paste, and negative control group. The roots were cultured for 21 days after being arranged vertically in sterilized glass tubes. Using Paper points Size 40, shaved dentin chips were gathered for bacterial culture. In order to measure the antibacterial activity, bacterial colony-forming units per milliliter (CFUs/ml) were used. The data were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at $p<0.05$. Statistical analysis was performed with R statistical analysis software version 4.4.2 for Windows

Results: The highest count was found in the control group, followed by nMgO, then the Calcium hydroxide group, while the lowest count was found in calcium hydroxide+ nMgO group.

Conclusion: Magnesium Oxide Nanoparticles plus Calcium hydroxide paste mixture was the most effective in suppressing the bacterial load of E. faecalis, after a 21-days incubation period.

KEYWORDS: Magnesium Oxide Nanoparticles, Nanoparticles in Endodontics, Intracanal medicament.

* MSc Student, Endodontic Department, Minia University, Egypt
** Assistant Professor, Microbiology & Immunology Department, Faculty of Pharmacy, Minia University, Egypt
Microbiology and Immunology Department, Faculty of Pharmacy, Deraya University, Minia, Egypt.
*** Assistant Professor, Department of Endodontics, Faculty of Dentistry, Minia University, El-Minia, Egypt
**** Microbiology and Immunology , University of Deraya , Minia , Egypt

INTRODUCTION

One bacterial species that is frequently seen in contaminated root canals is Enterococcus Faecalis, or E. faecalis. It promotes virulence factors that invade dentinal tubules and adhere to the root canal's dentin surface to form a biofilm, such as digestive enzymes and cytolysin. [1,2,3]

The complex structure of the root canal system makes it challenging to use traditional instrumentation techniques to achieve the best possible disinfecting. Root canals have been disinfected using a variety of techniques. Endodontic biofilm can be managed chemically by irrigation and mechanically through cleaning and shaping during biomechanical treatment.[4]

In order to eradicate the pathogens in root canals, intracanal medications have been considered an essential step. The most popular intracanal drug used in endodontics is calcium hydroxide ($Ca(OH)_2$) because of its antibacterial effects. The bacterial cell membrane, DNA, and protein structure are destroyed by its high alkalinity, which is brought on by the release of hydroxyl ions. Despite its benefits, E. faecalis is resistant to ($Ca(OH)_2$) high pH.

Incorporating nanoparticles into various dental materials has been promoted recently. The medication's antibacterial effectiveness was intended to be enhanced by the addition of nanoparticles. Because of their increased surface area, charge density, and polycationic/polyanionic nature, which allow for better contact with bacterial cells, nanoparticles have higher antibacterial efficacy. They can disperse antibacterial agents far into the dentin. The biocompatibility and antibacterial activity of inorganic nano metal oxides, even at low concentrations, have drawn increased interest. [1,5,6]

Numerous writers have addressed the use of nanoparticles in irrigation in their works. The antimicrobial properties of nano metal oxides as irrigant have been proven but further studies considering the ability of using nanomagnesium oxide as intracanal medication is required. Therefore, the aim of the present study was to evaluate the antibacterial effect of nano based intracanal medicaments (Magnesium Oxide nanoparticles) comparing to conventional Calcium hydroxide. The null hypothesis stated that there is no difference between using nMgO, $Ca(OH)_2$ or combination of them as intracanal medicaments (ICM) during root canal treatment in the reduction of the E. faecalis count.

MATERIALS AND METHODS

Ethical regulations

An ethical clearance obtained for the proposal of the research from the ethics and postgraduate committee of the faculty of Dentistry, Minia University in their session (Meeting no 106, Date:30/4/2024, Approved no 907) before carrying out the study.

An informed consent signed by the patient whose teeth used for the study before extraction.

Sample size calculation

The minimum total required sample size (n) was found to be (8) samples. The sample size was increased to account for possible failures during testing to be (80) samples (20 samples per group).

Selection of samples

Eighty uni-radicular human teeth that had been extracted for orthodontic or periodontal reasons were gathered. Preoperative radiographs in both buccolingual and mesiodistal directions were taken. Roots with Carious lesions, resorption, calcification and fracture were excluded.

Sample preparation:

All collected teeth were stored for two minutes in NaOCl 5.25% for surface disinfection, soft tissues dissolution, then the teeth's external root surfaces

were preserved in saline to avoid dehydration after being cleansed with a curette for removing calculus and periodontal tissues. Crowns were cut off until each root measured 15 mm.

The patency of each canal was assessed by inserting sterile ISO K-files size #15 (Micro Mega SA, Besancon, France) into the apical foramen and pushing back until the file flushed with the visible apical foramen, using the Protaper universal Rotary File System (DENTSPLY, Switzerland), all teeth were mechanically prepared in compliance with the manufacturer's instructions with the aid of E-connect S Wireless Endo-motor (Changzhou Sifary Medical Technology Co., Ltd) at speed 300 rpm and 1.5N.cm as a torque according to the instructions of manufacture.

Three milliliters of 2.5 percent sodium hypochlorite (NaOCl) were used to irrigate the samples in order to eliminate organic debris. One milliliter of 17% Ethylene Diamine Tetra Acetic acid was then added (EDTA Prevest Denpro Limited/ REF 50002/India) to remove inorganic debris. Finally, each sample were irrigated with 5 milliliters of distilled water using 30-gauge needle (Fanta dental. China) to get rid of any residual previous irrigants, then samples autoclaved for 20 min at 121 °C using steam air autoclave (Rundeer Control Equipment Co, Ltd) [7]

Preparation of microbial Culture:

E. faecalis strains were obtained from Reference laboratory of Egyptian University Hospitals -Cairo.

E. faecalis (ATCC 29212) was grown in brain heart infusion (BHI) agar at 37 °C until log phase.

Sample Classification:

All samples (n = 80) was allocated into four groups at random based on medication.

- Group A (n= 20): No medication (negative control)
- Group B (n= 20): Magnesium Oxide Nanoparticles paste (nMgO Paste).
- Group C (n= 20): Calcium hydroxide.
- Group D (n= 20): $Ca(OH)_2$ + nMgO Paste.

Infection of the root samples:

- E. faecalis log-phase culture (30 μL) was added to the root canals. Subsequently, the samples were incubated for 21 days to facilitate the formation of mature biofilms. (8) All procedures were conducted within a biosafety cabinet. (7)
- Every operation was completed at Deraya University's Faculty of Pharmacy at Minia.

Intra-canal medication:

Following the period of contamination, 3 ml of sterile saline were used to irrigate each specimen, and sterile paper points #40 (GAPADENT CO., LTD., TianJin City, P.R. China) were used to dry them

- **Group A:** No medication.
- **Group B:** Readymade nMgO gel was applied into each root canal using premixed syringe. The whole volume was delivered to ensure that the canal was filled.
- **Group C:** Calcium hydroxide Metapaste (MetaPaste, Meta Biomed, Seoul, Korea) was placed by the tip of the ready-made injectable paste to make sure that the medicament filled all the canal and got in contact with all walls.
- **Group D:** Metapaste (Meta Biomed) and magnesium oxide gel were added together in a 1:1 ratio to make the combined mixture easier to handle and apply into the canals using a sterile plastic syringe with the tip of the prefabricated injectable paste was subsequently modified to the working length.

Methods of evaluation:

After sealing the canal orifices with Teflon and placing the specimens in safety cabinets the samples were incubated for 7 days at 37°C. The Teflon seal was taken off following the incubation time. Protaper universal Rotary File System F4 was utilized at 300 RPM and 2 N.cm torque to remove the medication. Sterile saline solution was used to irrigate the root canals, and a sterilized paper points # 40 were used to dry them. Sterile #40 H-files are used to harvest dentin from the root canals of every specimen in each group, along the all working length of the root canal.

Each file was placed in a sterilized Eppendorf test tube containing 1 milliliter of sterile saline and vortexed for 30 seconds then 20 µl of this content was plated on (BHI) agar for 48 hours to count the colonies of E. faecalis.

Colony forming units (CFU) were used to count and record growing colonies.[9] every outcome was gathered, totaled, and subjected to statistical analysis

Statistical analysis:

The standard deviation (SD), mean with 95% CIs, minimum (min.) and maximum (max.) values were used to display the numerical data. By examining the data distribution and applying the Shapiro-Wilk and Levene tests, respectively, they were investigated for normality and variance homogeneity. The distribution was skewed, and the Box-Cox transformation was used to normalize the data. After the transformation, all the assumptions were validated. Tukey's post hoc test was used after a one-way ANOVA to analyze the results. A significance threshold of $p<0.05$ was established. R statistical analysis software, version 4.4.2 for Windows, was used to conduct the statistical analysis.*

RESULTS

Intergroup comparisons, mean and standard deviation values of transformed antibacterial efficacy (CFU/ml) $\times 10^4$ are presented in Table (1) and in Figure (1)

The groups differed significantly from one another ($p<0.001$). nMgO (1.48±0.26) (CFU/ml) 10^4, Ca (OH) (1.32±0.09) (CFU/ml) 10^4, and the control group (3.37±0.20) (CFU/ml) 10^4 had the highest count, whereas Ca $(OH)_2$ and nMgO (1.20±0.20) (CFU/ml) 10^4 had the lowest count. The group acting as a control had significantly higher numbers than the other groups, according to post hoc pairwise comparisons ($p<0.001$). They also demonstrated that nMgO had a substantially larger count than Ca $(OH)_2$ & nMgO ($p<0.001$).

TABLE (1) Intergroup comparisons, mean and standard deviation values of antibacterial efficacy (CFU/ml) $\times 10^4$.

Antibacterial efficacy (CFU/ml) *10^4 (Mean±SD)				p-value
Ca $(OH)_2$	nMgO	Ca $(OH)_2$ & nMgO	Control	
1.32 ± 0.09^{BC}	1.48 ± 0.26^{B}	1.20 ± 0.20^{C}	3.37 ± 0.20^{A}	**<0.001***

Values with different superscripts within the same horizontal row are significantly different *; significant (p<0.05).

* R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

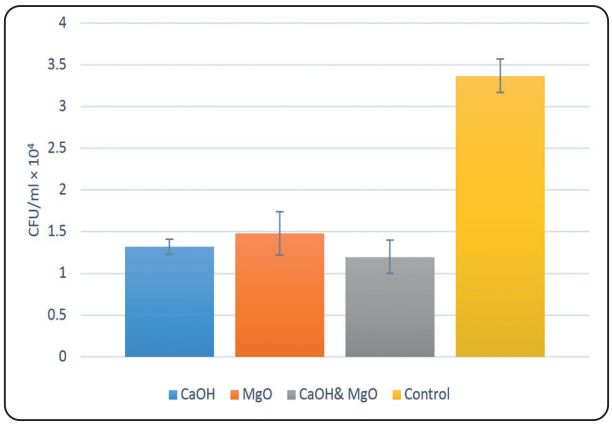

Fig. (1) Bar chart showing mean and standard deviation (error bars) of antibacterial efficacy (CFU/ml) × 10^4.

DISCUSSION

Eliminating bacteria from canals and preventing infection and reinfection by treating the canal chemically and mechanically are the primary objectives of root canal therapy. The complicated architecture of the canals and chronic, long-lasting infections that allow bacteria to infiltrate deeply into the dentinal tubules are a couple of situations that can make achieving this goal challenging.[1,3] It was found that E. faecalis predominated in the majority of endodontically treated patients that failed.[10]

The prevalence of E. faecalis, an opportunistic bacterium, in cases of chronic periapical diseases ranges from 29% to 77%.[11] After 24 hours, it can form biofilms,[12] but three weeks is enough to produce a more stable and developed biofilm. In contrast to immature biofilms, Yang discovered that mature biofilms are less susceptible to disinfection. To ensure the presence of a mature biofilm, the E. faecalis biofilm was grown in this investigation over a period of three weeks.[13]

Such bacterial species are eliminated using a variety of methods, particularly in retreatment situations. In order to decrease the bacterial load, intracanal medications are injected into the canal and kept there for a predetermined amount of time. The intracanal medicaments that are readily used are calcium hydroxide, chlorohexidine and antibiotic pastes. Calcium hydroxide ($Ca(OH)_2$) paste is a widely used intracanal medicament.[14]

E. faecalis has agreat resistance to alkaline media.[15] As an intracanal medication, normal sized $Ca(OH)_2$ shown mild antibacterial activity against E. faecalis in earlier trials.[16,17,18]

There have been numerous applications of nanotechnology in dentistry. It was utilized in irrigation materials, medications, and sealers in endodontics. The goal of turning regular medication particles into nanoparticles was to make the materials denser and more capable of penetrating deeply into the dentinal tubules for improved antibacterial activity.[19]

In this study, the antimicrobial effects of calcium hydroxide, magnesium oxide nanoparticles, and ($Ca(OH)_2$ + nMgO) on the E. faecalis biofilm were compared.

The samples were selected, cleaned and each root was reduced to a length of 15 mm for Standardization.[7]

Root canals were shaped up to F4 rotary file to create a large reservoir for the irrigant solution.[20] The samples underwent irrigation with sodium hypochlorite (NaOCl), followed by 17% Ethylene Diamine Tetra Acetic acid to effectively remove organic and inorganic debris from the root canals.[21] The samples inoculated with E. faecalis and incubated on BHI (specific media for the growth of the tested microorganisms) for 21 days to facilitate the formation of mature biofilms.[22]

Subsequently, for uniformity and ease of application and removal, all of the components utilized in this investigation have been used as paste. In contrast to nano-silver, nano-TiO2, nano-copper, and other kinds of nanomaterials and bactericides, magnesium oxide nanoparticles are easily made from easily accessible and reasonably priced precursors, which is why we used them in our study.[23,24]

This study used a 7-day incubation period, as it is the minimum time required for medicaments to function effectively as an inter-appointment medication. [25] Since the physiologic saline has no antibacterial properties, it was selected for medicament removal. [26,27]

Using colony forming units (CFU), the antibacterial efficacy of the investigated intracanal medications was assessed. By assessing the number of colonies after the test, CFU is the most practical and reliable technique for accurately determining the differences the antibacterial efficacies of various materials. [9] The CFU/ml result was calculated by multiplying the number of visible colonies on an agar plate by the dilution factor. [28] According to the literature, this was the most effective sample collecting technique for ensuring adequate collection of bacteria found on all dentin surfaces. [9,29]

The finding rejects the null hypothesis that there is no difference between using nMgo, Ca $(OH)_2$ or combination of them as ICM during root canal treatment in the reduction of the E. faecalis count.

The results showed that nMgo paste reduced the number of colony forming units per milliliter (CFU/ml) of E. faecalis. Although, nMgO group showed a higher mean (1.48) (CFU/ml) compared to the Ca $(OH)_2$ group (1.32) the difference was not statistically significant, this finding came in agreement with Yousefshahi who compared the effects of metal oxides nanoparticles on the inhibitory effects of calcium hydroxide based on E. faecalis species showed that magnesium oxide had the lowest inhibition zone diameter.[39] Furthermore, Souror showed that nMgO had the least effect in root canal cleanliness but still has antibacterial properties, which can contribute to the disinfection and canal cleanliness of the root canal system.[30]

These outcomes could be brought on by the generation of superoxide anions (O_2-), hydroxyl radicals (•OH), and reactive oxygen species (ROS), which could damage DNA and bacterial cell membrane. [31]

Additionally, Jin showed that nMgO treatments cause the cell membrane to distort and break down, allowing intracellular contents to leak out and bacterial cells to die. As a result, nMgO was discovered to exhibit antibacterial activity against both bacteria and fungi. [32]

When Ca $(OH)_2$ is used alone as an intracanal medication against E. faecalis, its antibacterial efficiency is superior to that of MgO. This may be due to Ca $(OH)_2$ has a physical barrier and its hydroxyl ions denaturize DNA and proteins. [33,34]

On the other hand, Tewfik who evaluated the efficacy of an aqueous solution of magnesium oxide nanoparticles and its ultrasonic activation on root canal E. faecalis biofilm showed that MgO in the nano form exhibits superior antibacterial action these results may attributed to using different methodology.[35] Additionally Monzavi stated that nMgO aqueous solutions represent promising antimicrobial activity both in vitro and ex vivo with minimal toxicity this might be due to superoxide anions formed on the microbial cell surface were shown to be bactericidal when magnesium oxide was present in a hydrous shape. [36]

The effectiveness of calcium hydroxide against E. faecalis is not very noticeable. [37] But when nMgO is added to calcium hydroxide, the medication's antibiofilm effectiveness against E. faecalis is much enhanced.

The idea of adding nanoparticles to current medications, especially calcium hydroxide, to enhance its qualities has been studied. Antibacterial nanoparticles and calcium hydroxide have been proposed to work in conjunction to promote synergistic effects that would improve their antibacterial properties.[38] According to the results of the previous studies we used a combination of Ca $(OH)_2$ and nMgO. Our results showed that the mean was (1.20) which revealed that Ca $(OH)_2$ in combination with nMgO shown superior efficacy against E. faecalis compared to Ca $(OH)_2$ alone.

This finding came in agreement with Yousefshahi Who stated that the combination of nanoparticles with calcium hydroxide could significantly create an inhibition zone larger than calcium hydroxide alone. [39] Furthermore, at three-week intervals, Teja assessed that Ca $(OH)_2$ in combination with nanoparticles was more efficient against E. faccalis than Ca $(OH)_2$ alone[8] This might be due to the particles that are positively charged interact aggressively with the negative-charged bacterial cell walls and spores due to their enormous surface area.[40] They also have antibacterial properties against viruses and bacteria, both gram-positive and gram-negative.[41] the main target of metal oxide nanoparticles is the bacterial cell wall, which is composed of surface proteins that allow components like teichoic acid and polysaccharides that protect against external factors and host defense to attach and colonize.[42,43]

On the other hand, Tewfik et al., 2022 who evaluated the Efficacy of an aqueous solution of magnesium oxide nanoparticles and its ultrasonic activation on root canal E. faecalis biofilm showed that MgO in the nano form exhibits superior antibacterial action these results may attributed to using different methodology.[35] Additionally, Monzavi et al., 2015 stated that nano-MgO aqueous solutions represent promising antimicrobial activity both in vitro and ex vivo with minimal toxicity these results may be due to magnesium-oxide nanoparticles were found to be bactericidal when present in a hydrous configuration as a result of activity of superoxide anions established on the microbial cell surface. [9]

The present study has focused specifically on E. faecalis, highlighting the need for more research in multispecies biofilm, since endodontic biofilm is composed of a wide variety of microbial species. Nanoparticle synthesis remains a restriction and is not commonly used in therapies. In endodontics, there is currently little clinical application of disinfection based on nanoparticles. Additional research examining nMgO's capacity to dissolve pulpal and necrotic tissues, penetrate dentinal tubes, and remove the smear layer will be required to clarify additional facets of its antibacterial activity.

CONCLUSIONS

Under the limitation of the current study the following can be concluded: in single-rooted teeth, when compared to either calcium hydroxide or nMgO alone, the combination was the most successful in reducing the bacterial load of E. faecalis after 7-days incubation period but not statistically significant in comparison to calcium hydroxide alone.

REFERENCES

1. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod. 2014;40(2): 285-290.

2. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(1):86-93. doi:10.1016/S1079-2104(98)90404-8

3. Love RM. Enterococcus faecalis - A mechanism for its role in endodontic failure. Int Endod J. 2001;34(5):399-405. doi:10.1046/j.1365-2591.2001.00437.x

4. Gomes BPFA, Francisco PA, Godoi Jr EP, et al. Identification of culturable and nonculturable microorganisms, lipopolysaccharides, and lipoteichoic acids from root canals of teeth with endodontic failure. J Endod. 2021;47(7):1075-1086.

5. Shrestha A, Kishen A. Antibacterial Nanoparticles in Endodontics: A Review. J Endod. 2016;42(10):1417-1426. doi:10.1016/j.joen.2016.05.021

6. Padovani GC, Feitosa VP, Sauro S, et al. Advances in Dental Materials through Nanotechnology: Facts, Perspectives and Toxicological Aspects. Trends Biotechnol. 2015;33(11):621-636. doi:10.1016/j.tibtech.2015.09.005

7. Ugalmugale SR, Bohora AA, Patel PA, Sharma V, Sengupta S, Sharma SM. Comparative evaluation of antibacterial

efficacy of nitrofurantoin, chitosan, and calcium hydroxide in combination with propylene glycol as an intracanal medicament against endodontic pathogen–An in vitro study. Journal of Conservative Dentistry and Endodontics. 2024;27(8):801-806.

8. Teja KV, Janani K, Srivastava KC, et al. Comparative evaluation of antimicrobial efficacy of different combinations of calcium hydroxide against Enterococcus faecalis. BMC Oral Health. 2023;23(1):1-10. doi:10.1186/s12903-023-03552-4
9. Monzavi A, Eshraghi S, Hashemian R, Momen-Heravi F. In vitro and ex vivo antimicrobial efficacy of nano-MgO in the elimination of endodontic pathogens. Clin Oral Investig. 2015;19:349-356.
10. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal. 2019;24(3):e364.
11. Shorey P, Sidhu K, Bhushan J, Chhibber S, Bhardwaj SB. Ex-Vivo evaluation of Nano-MgO in the elimination of Endodontic pathogen-E. faecalis. IP Indian Journal of Conservative and Endodontics. 2022;6(4):222-227.
12. Lima RKP, Guerreiro-Tanomaru JM, Faria-Júnior NB, Tanomaru-Filho M. Effectiveness of calcium hydroxide-based intracanal medicaments against Enterococcus faecalis. Int Endod J. 2012;45(4):311-316.
13. Elkillany R, Sabet N, Fakhr M. The Antimicrobial Efficacy of Nanoparticles Intracanal Medicaments Against Enterococcus Faecalis Biofilm. Egypt Dent J. 2022;68(2):1789-1796.
14. Siqueira Jr JF, de Uzeda M. Influence of different vehicles on the antibacterial effects of calcium hydroxide. J Endod. 1998;24(10):663-665.
15. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta□analysis. Int Endod J. 2007;40(1):2-10.
16. Padovani GC, Feitosa VP, Sauro S, et al. Advances in Dental Materials through Nanotechnology: Facts, Perspectives and Toxicological Aspects. Trends Biotechnol. 2015;33(11):621-636. doi:10.1016/j.tibtech.2015.09.005
17. Velasco-Loera N, De Alba-Vazquez Y, Garrocho-Rangel A, Gonzalez-Amaro AM, Flores-Reyes H, Pozos-Guillen AJ. Comparison of the antibacterial effect of modified 3-mix paste versus Ultrapex over anaerobic microorganisms from infected root canals of primary teeth: An in vitro study. Journal of Clinical Pediatric Dentistry. 2012;36(3):239-244.
18. Hiraishi N, Yiu CKY, King NM, Tagami J, Tay FR. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. J Endod. 2010;36(6):1026-1029.
19. Chogle S, Kinaia BM, Goodis HE. Scope of nanotechnology in endodontics. Nanobiomaterials in Clinical Dentistry. Published online 2019:517-539.
20. Lei HE, Haibing Y. Protaper nickel-titanium file versus OrodekaPle-xv nickel-titanium file in the preparation of root canal of molars. Journal of Clinical Medicine in Practice. 23(9):38-40.
21. Ugalmugale SR, Bohora AA, Patel PA, Sharma V, Sengupta S, Sharma SM. Comparative evaluation of antibacterial efficacy of nitrofurantoin, chitosan, and calcium hydroxide in combination with propylene glycol as an intracanal medicament against endodontic pathogen–An in vitro study. Journal of Conservative Dentistry and Endodontics. 2024;27(8):801-806.
22. Attia DA, Farag AM, Afifi IK, Darrag AM. Antimicrobial effect of different intracanal medications on various microorganisms. Tanta dental journal. 2015;12(1):41-47.
23. Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Reproductive and developmental toxicity studies of manufactured nanomaterials. Reproductive Toxicology. 2010;30(3):343-352.
24. Koper OB, Klabunde JS, Marchin GL, Klabunde KJ, Stoimenov P, Bohra L. Nanoscale powders and formulations with biocidal activity toward spores and vegetative cells of bacillus species, viruses, and toxins. Curr Microbiol. 2002;44:49-55.
25. Valverde ME, Baca P, Ceballos L, Fuentes MV, Ruiz-Linares M, Ferrer-Luque CM. Antibacterial efficacy of several intracanal medicaments for endodontic therapy. Dent Mater J. 2017;36(3):319-324.
26. Javidi M, Afkhami F, Zarei M, Ghazvini K, Rajabi O. Efficacy of a combined nanoparticulate/calcium hydroxide root canal medication on elimination of E nterococcus faecalis. Australian Endodontic Journal. 2014;40(2):61-65.
27. Yehia TYAM, El-Ashry S, El-Batoty K, El-Hady S. Efficiency of Triple Antibiotic Mixture and Propolis as Intracanal Medication in Revascularization process in immature apex: A clinical study. Global Journal of Medical and Clinical Case Reports. 2019;6(2):19-25.

28. Vianna ME, Horz H, Conrads G, Feres M, Gomes B. Comparative analysis of endodontic pathogens using checkerboard hybridization in relation to culture. Oral Microbiol Immunol. 2008;23(4):282-290.

29. De Almeida J, Cechella BC, Bernardi AV, de Lima Pimenta A, Felippe WT. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian Journal of Dental Research. 2018;29(3):347-351.

30. Souror KK, El Gendy A, Nabil M. The Efficacy of Nano Magnesium Oxide and Nano Silver Fluoride on Canal Cleanliness and Smear Layer Removal (An In Vitro Study). Egypt Dent J. 2024;70(3):2853-2866.

31. Yousefshahi H, Aminsobhani M, Shokri M, Shahbazi R. Anti-bacterial properties of calcium hydroxide in combination with silver, copper, zinc oxide or magnesium oxide. Eur J Transl Myol. 2018;28(3).

32. Jin T, He Y. Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. Journal of Nanoparticle Research. 2011;13:6877-6885.

33. Adl A, Shojaee NS, Motamedifar M. A Comparison between the Antimicrobial Effects of Triple Antibiotic Paste and Calcium Hydroxide Against Entrococcus Faecalis. Vol 7.; 2012.

34. Zehnder M, Luder HU, Schätzle M, Kerosuo E, Waltimo T. A comparative study on the disinfection potentials of bioactive glass S53P4 and calcium hydroxide in contra-lateral human premolars ex vivo. Int Endod J. 2006;39(12):952-958.

35. Tewfik H, Nabeel M. The Efficacy of an Aqueous Solution of Magnesium Oxide Nanoparticles and Its Ultrasonic activation on Root Canal Enterococcus Faecalis Biofilm. In Vitro Study. Journal of Fundamental and Clinical Research. 2022;2(2):112-128.

36. Chandak PG, Chandak MG, Relan KN, Chandak M, Rathi C, Patel A. Nanoparticles in endodontics-a review. J Evol Med Dent Sci. 2021;10(13):976-982.

37. Rao MH, Rajkumar K, Pavithra G, et al. Antibiofilm efficiency of silver and copper nanoparticle incorporated calcium hydroxide as an intracanal medicament: An in vitro study. J Oral Biol Craniofac Res. 2025;15(2):319-324.

38. Bukhari SH, Abraham D, Mahesh S, Bukhari S. Antimicrobial Effects of Formulations of Various Nanoparticles and Calcium Hydroxide as Intra-canal Medications Against Enterococcus faecalis: A Systematic Review. Cureus. 2024;16(9).

39. Yousefshahi H, Aminsobhani M, Shokri M, Shahbazi R. Anti-bacterial properties of calcium hydroxide in combination with silver, copper, zinc oxide or magnesium oxide. Eur J Transl Myol. 2018;28(3).

40. Huang L, Li DQ, Lin YJ, Wei M, Evans DG, Duan X. Controllable preparation of Nano-MgO and investigation of its bactericidal properties. J Inorg Biochem. 2005;99(5):986-993. doi:10.1016/j.jinorgbio.2004.12.022

41. Leung YH, Ng AMC, Xu X, et al. Mechanisms of antibacterial activity of mgo: Non-ros mediated toxicity of mgo nanoparticles towards escherichia coli. Small. 2014;10(6):1171-1183. doi:10.1002/smll.201302434

42. Zhang K, An Y, Zhang L, Dong Q. Preparation of controlled nano-MgO and investigation of its bactericidal properties. Chemosphere. 2012;89(11):1414-1418. doi:10.1016/j.chemosphere.2012.06.007

43. Barge P, Gugawad S, Devendrappa SN, Hadakar S, Taur SM. Comparative Evaluation of Nano Inorganic Metal Oxides as Intracanal Medicaments for Cytotoxicity and Antimicrobial Activity in the Root Canal System. Int J Clin Pediatr Dent. 2023;16(Suppl 2):168.