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Evaluation of Antibacterial Properties and Solubility of Modified Metapex with Zinc Oxide Nanoparticles: An In Vitro Study

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ABSTRACT

Background and Objective: Pulpectomy plays a critical role in maintaining primary teeth until they naturally exfoliate, particularly in cases where the pulp is infected or necrotic. Metapex is a widely used root canal filling material for such treatments.

Methods: This study investigates the effect of incorporating zinc oxide nanoparticles (ZnO NPs) into Metapex on its antibacterial efficacy and solubility. Four groups were evaluated: a control group with Metapex alone and three experimental groups with Metapex modified with 1%, 3%, and 5% concentrations of ZnO NPs. The modified pastes were thoroughly homogenized. Antibacterial activity was tested against Enterococcus faecalis using the agar well diffusion method, and solubility was assessed by calculating weight change before and after immersion in distilled water.

Results: The results showed significant improvements in antibacterial activity with higher concentrations of ZnO NPs ($p < 0.001$). The 5% ZnO group produced the largest inhibition zone (11.42 ± 0.74 mm), and both 3% and 5% concentrations showed significantly greater antibacterial effects than the control. In terms of solubility, pure Metapex exhibited the highest rate (35.17 ± 0.59), while the addition of ZnO NPs resulted in a concentration-dependent decrease in solubility: 32.93 ± 2.11 for 1%, 27.77 ± 4.37 for 3%, and 24.03 ± 1.97 for 5% ($p = 0.003$).

Conclusion: The integration of ZnO NPs into Metapex enhances its antibacterial capabilities while improving its physical stability. A 5% concentration appears to offer the most effective balance, making it a promising enhancement for pulpectomy procedures.

Keywords: Antibacterial activity, Enterococcus faecalis, Metapex, Solubility, Zinc oxide nanoparticles

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INTRODUCTION

Endodontic treatment involving complete extirpation of pulp tissue, termed pulpectomy, is commonly applied to primary teeth with irreversibly pulpitis or with a necrotic pulp. Following chemomechanical root canal preparation, a biocompatible and resorbable material should be applied to achieve healing and to deliver tooth retention within the oral cavity until its spontaneous exfoliation occurs.[1]

Root treatment of primary molars with nonvital pulps is a great challenge to the pediatric dentist, primarily because the canal structure is very complex. Such complexity encourages the growth of microorganisms so that they penetrate into lateral canals, apex delta formations, and dentinal tubules.[2] Pulpectomy with the removal of the damaged pulp tissue decreases the microbial load, but to increase the efficacy of pulpectomy, a disinfection of the pulp chamber is also required with an obturating material that has very good antibacterial properties.[3] Some of the obturation materials have been applied to the primary teeth, and zinc oxide eugenol (ZOE) has been the time-honored favorite. Moreover, the compounds of iodoform and calcium hydroxide have also gained quite a bit of popularity. ZOE was the traditional obturating agent of primary dentition till now. It was the first to be advocated. But its resorptive rate is relatively slow compared to that of the deciduous tooth. ZOE can also cause irritation to periapical areas, cause necrosis of the cementum and bone, and also upset the course of eruption of the tooth about to come.[4]

Herman first introduced calcium hydroxide in 1920. A significant limitation of this material is its tendency to deplete from canals more rapidly than the physiological process of root resorption, despite its antiseptic and osteoconductive properties. Hydroxyl ions inactivate bacterial cytoplasmic membrane enzymes, causing antimicrobial effects.[5] Metapex (METABIOMED) and Vitapex (Neo Dental Chemical Products Co., Ltd, Tokyo, Japan) are provided in prefilled syringes, allowing for direct placement into the canals and extrusion through simple pressure.[6] Rapid Metapex and Vitapex resorption creates voids inside the canal, which eventually cause a hollow tube to form.[7] Certain qualities, particularly antibacterial activity, may be improved by adding nanoparticulated materials to a variety of materials. Both microscale and nanoscale versions of zinc oxide (ZnO) are now being investigated as antibacterial agents. According to research, ZnO nanoparticles are more effective at fighting bacteria than their microparticle counterparts.[10] Additionally, it is known that applying nanoparticulated zinc oxide increases the antibacterial efficacy of several products, especially against biofilms produced by Enterococcus faecalis and Staphylococcus aureus.[8]

Despite the clinical success of Metapex, its rapid resorption rate often leads to the formation of voids, potentially compromising the long-term success of the treatment. While ZnO nanoparticles have shown promise in enhancing antimicrobial efficacy, there is a lack of evidence regarding the optimal concentration of these nanoparticles when incorporated into Metapex to balance both antibacterial activity and solubility. Therefore, it is necessary to investigate whether modifying Metapex with specific concentrations of ZnO nanoparticles can address these limitations. The antibacterial effect of two common obturation materials of primary teeth was assessed in this study: a combination of ZnO nanoparticles and calcium hydroxide with iodoform (Metapex) against Enterococcus faecalis bacteria.

Aim of this study

The aim of the study was:

To assess and compare the antibacterial efficacy and solubility of modified Metapex with the addition of ZnO nanoparticles at different concentrations (1%, 3%, 5%).

METHODS

This study was conducted in vitro, over a duration of 6 months. For this study, one obturating material (Metapex) with zinc oxide nanoparticles was used as experimental material; the compositions and manufacturers are shown in Table 1 and Figure 1

Figure 1. (A) Zinc oxide nanoparticles (Nanografi, Teknokent Çankaya/Ankara TURKEY), (B) Metapex (METABIOMED CO., LTD. Korea)

Table 1. Materials that Were Used in the Study

No	Material	Composition	Manufacturer
1	Metapex	Calcium hydroxide 36%, Iodoform 37%, Polydimethylsiloxane 27%	METABIOMED CO., LTD. Korea. LOT: MXP24-1151
2	Zinc oxide nanoparticles	ZnO Nanopowder/Nanoparticles, Purity: 99.5%, Size: 18 nm	Nanografi Turkey ODTÜ Teknokent No:13/1-1 06800 Çankaya/ Ankara TURKEY

Preparation of experimental Metapex and ZnONPs Mixtures

A ratio and proportion equation was employed to combine Metapex with zinc oxide nanoparticles to prepare the test mixtures. The Metapex syringe contains 2.2 grams of paste, which serves as the base material. To achieve the desired weight percentages of zinc oxide nanoparticles, the following additions were made:

- For the 1% ZnO mixture, 0.022 grams of ZnO powder were added to the total 2.2-gram Metapex paste.
- For the 3% ZnO mixture, 0.066 grams of ZnO powder was added to the 2.2-gram Metapex paste.
- For the 5% ZnO mixture, 0.11 grams of ZnO powder was added to the 2.2-gram Metapex paste.

The mixing procedure involved placing the calculated amount of ZnO powder on a sterilized paper pad and mixing it with the Metapex paste using a sterilized spatula. The mixture was then transferred to a sterilized empty test tube and homogenized by using an ultrasonic sonicator, as shown in Figure 2.

Figure 2. Mixing of Metapex with ZnO Nanoparticles by ultrasonic sonicator

Chemical characterization of tested materials

The effect of filler addition particles on the chemistry of Metapex sealer was determined by using Fourier Transform Infrared Spectroscopy FTIR (PIKE Technologies, Madison, WI, USA) Figure (3, A), it is a powerful tool for analyzing the chemical interactions and bonding in mixtures like calcium hydroxide (Ca(OH)) with zinc oxide (ZnO) nanoparticles, FTIR was done in Salahaddin University, College of Science, for metapex alone, zinc oxide nanoparticles alone and when its mixed together.[9]

Scanning electron microscopy (SEM, Quanta 450) analysis was performed to confirm the homogenous distribution of the ZnO nanoparticles in the Metapex sealers, Figure (3, B). This was conducted at Soran University, Department of Scientific Research Center. SEM was used for the control, and each testing group.[9]

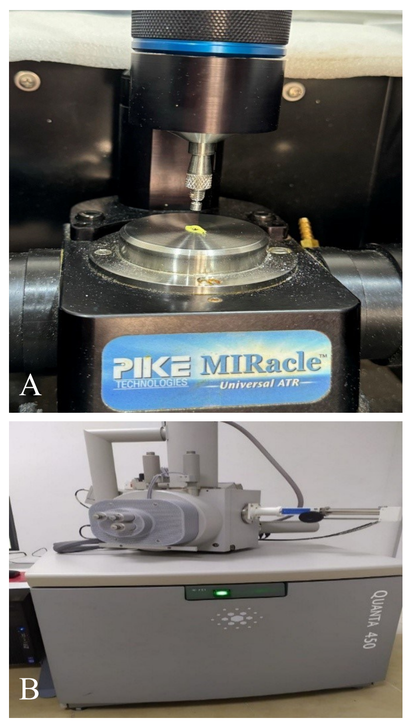

Figure 3. (A) Fourier Transform Infrared (FTIR) Spectroscopy, (B) SEM Quanta 400

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Anti-bacterial test

The antibacterial experiment was comprised of 24 samples that were split into four groups comprising six samples each. In a study to understand the influence of antibacterial action, the agar well diffusion test (ADT) was adopted. With the typical Enterococcus faecalis (ATCC 29212) strain, antibacterial action was assessed on the obturating materials. Microorganism strains were cultured in Thioglycolate broth for 24 hours. A spectrophotometer set to the 0.5 McFarland scale was used to standardize the suspensions. Using a sterile swab, a suspension of E. faecalis was transferred and spread on Muller-Hinton Agar plates and left to grow at 37°C for 24 hours. A clean glass tube was then used to make four agar wells (6 mm wide and 4 mm deep) in each petri dish. The wells were filled with the following: Group 1 was Metapex as a control group; Group 2 was Metapex with 1% ZnO; Group 3 was Metapex with 3% ZnO nanoparticles; and Group 4 was Metapex with 5% ZnO nanoparticles. Before placing the material into the wells of each petri dish, it was mixed according to the manufacturer's directions until achieving a uniform consistency. All the ingredients used for obturation were placed into the wells in the appropriate groupings. Each antibacterial test was performed in triplicate on three separate days (n=6 independent experiments). Within each experiment, three replicates were tested (n=6).

For 24 hours, all the petri dishes were maintained at 37°C. After incubation, the areas where bacterial growth was inhibited were measured at the radical zone with a transparent ruler.

Solubility test

The study examined solubility by measuring how much the weight of the material changed. Initially, the 3D models were fabricated using a Creality Halot Sky LCD-based resin 3D printer (Shenzhen Creality 3D Technology Co., Ltd., China), in combination with Creality Standard Photopolymer Resin was used to make 12 resin cylinders that were 1 mm thick, 2.5 mm wide, and 3 mm high (Figure 4, A),[10] To seal one end of the cylinders, adhesive waxes were used. Then, a separating material was placed inside the cylinders using a micro -brush to facilitate the removal of samples from the mold. There were four groups of cylinders, each with three cylinders (n=3 per group).[11] Pulpal pastes were prepared by adding zinc oxide nanoparticles to Metapex with a hand spatula and increasing the percentage of zinc oxide nanoparticles to obtain three different concentrations (1%, 3%, 5%). The solubility was determined by obtaining the percentage weight change of the material. Molds were filled with pulpal paste, and they were initially charged with wet cotton balls at room temperature for one day. Then they were put in the incubator (Memmert GmbH, Western Germany) at 37°C with saturated moisture for five further days.[10,12] The incubator was taken out of the samples. The samples were kept in a desiccator (Stony Lab Glass Desiccator, Nesconset, NY, USA) for 24 hours before the first weighing (Figure 4, B). We used a Sartorius BP 221S analytical balance (Sartorius AG, Germany) with a resolution of 0.0001g to weigh each sample. The first weight was obtained using the average value of two readings. After 24 hours, three discs of each pulpal paste were removed from the distilled water and then were again weighed using a desiccator with an overnight drying time. We calculated the percentage change in weight and referred to it as the solubility of pulpal pastes. Using the following equation, we found out how soluble the substance was.[9]

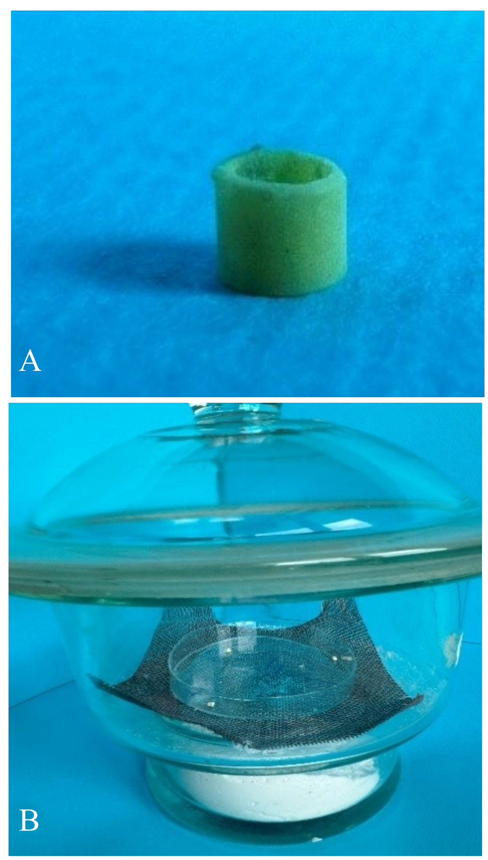

Figure 4. (A) Cylinders mold made of resin designed by CAD-CAM, (B) Desiccator (Stony Lab Glass Desiccator, Nesconset, NY, USA)

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Both inhibition zones and weight changes were blinded to the experimental group assignments to reduce measurments bias.

Statistical Analysis

Data was analyzed with SPSS software version 27. A Shapiro-Wilk test analyzed solubility and zone of inhibition data to assess normality. If the outcome of the test of normality was a normal distribution, parametric tests were utilized. ANOVA compared differences across groups within a context of normally distributed data. If the data were not normally distributed, non-parametric tests were utilized. A Kruskal-Wallis test was utilized to establish group differences within non-normally distributed data. Tukey's test was utilized to make pairwise comparisons with normally distributed solubility data, and a Mann-Whitney test was utilized with zone of inhibition data. Statistical significance was established at a p-value cut-off point of less than 0.05.

RESULTS

FTIR Spectroscopy

FTIR analysis confirmed functional groups in ZnO, Metapex and their mixture. ZnO showed O-H stretching at ~3383 cm-1 and ZnO at 460 cm-1. Metapex displayed O-H (3641 cm-1), C-H (2962 cm-1), and phosphate/carbonate bands (1257,1082,1012 cm-1). In the ZnO-Metapex mixture, key peaks from both components remained (eg., Zn-O at 472cm-1, Metapex peaks at 1259 and 1012 cm-1) indicating no major chemical interaction, suggesting a physical blend as shown in Figure 5.

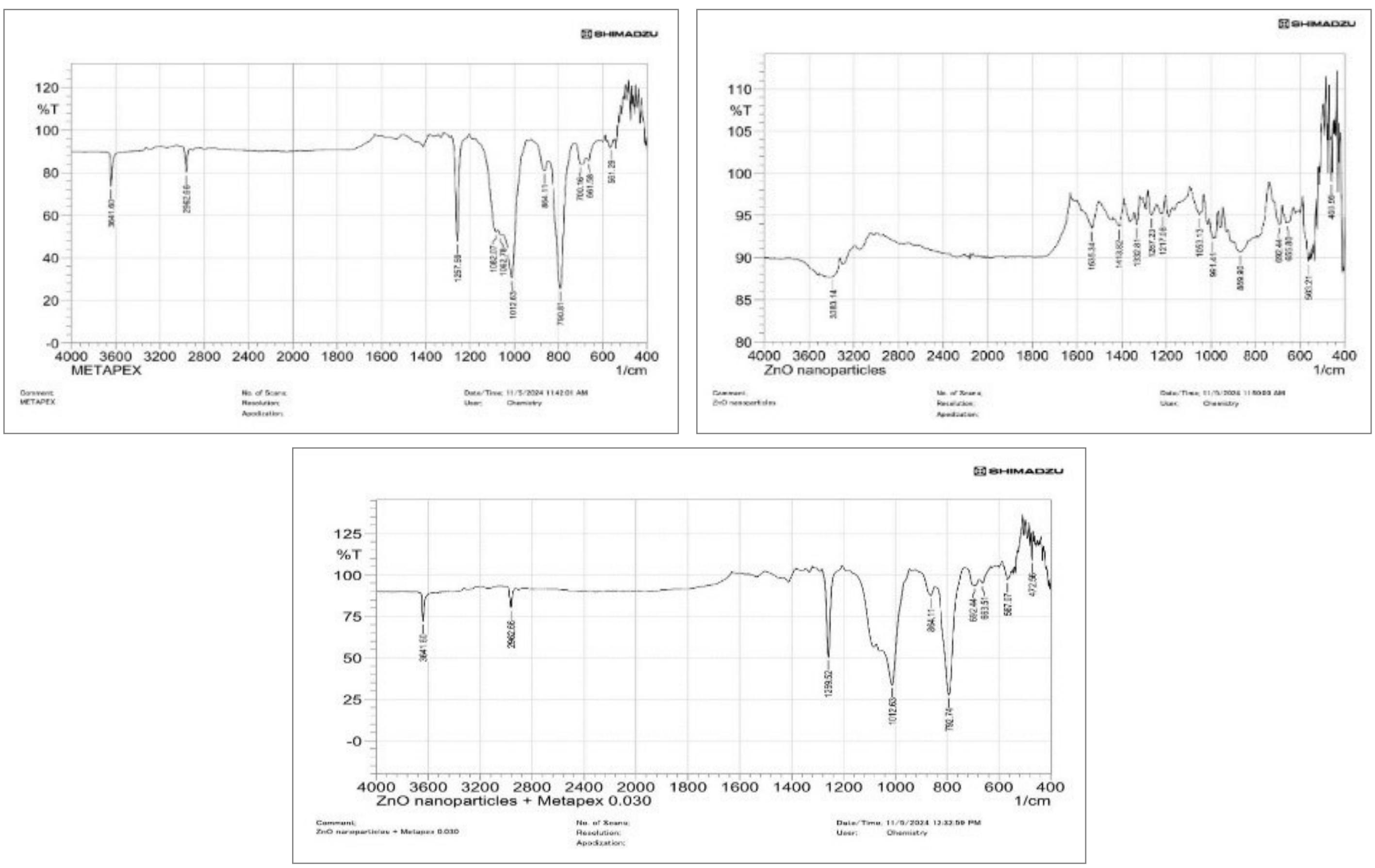

Figure 5. FTIR spectra investigated, (A) Metapex, (B) ZnO Nanoparticle, and (C) Experimental material (Mixture of Metapex and ZnO nanoparticles)

SEM

SEM images of experimental material showed individual ZnO Nanoparticles that are embedded in the Metapex sealer and there was homogenous distributions of the filler inside the sealer, the use of ultrasonication was to ensure a uniform distribution of the nanoparticles in the sealants, as shown in Figure 6.

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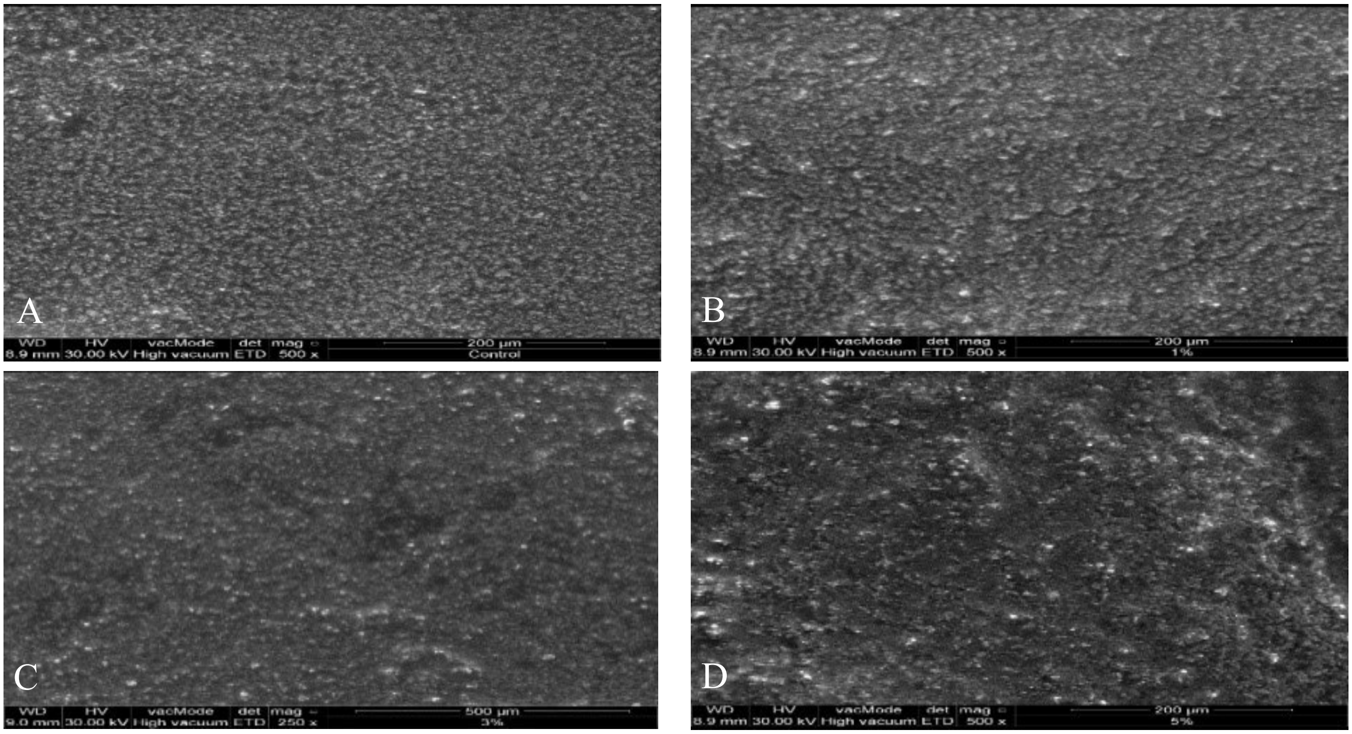

Figure 6. SEM photomicrograph of homogenous distribution of ZnO powder in Metapex sealer, (A) Metapex, (B) 1% , (C) 3% , (D) 5%.

Antibacterial test

The normality of the solubility and Zone of Inhibition data for different combinations of Metapex with various ZnO concentrations (0%, 1%, 3%, and 5%) was assessed using the Shapiro-Wilk test. Solubility data for all groups (Metapex, Metapex + 1% ZnO, Metapex + 3% ZnO, and Metapex + 5% ZnO) are normally distributed. However, the Zone of Inhibition data did not follow a normal distribution. Based on these results, for further analysis, parametric tests (ANOVA) were used for the Solubility variable, while for the Zone of Inhibition data non-parametric tests (Kruskal-Wallis) were considered. Antimicrobial action of Metapex added with various concentrations of ZnO nanoparticles was assessed through zone of inhibition Table 2 and illustrated in Figure 7. There was observed a considerable degree of variation among the groups ($p < 0.001$, Kruskal-Wallis test). An average zone of inhibition of 6.17 ± 0.26 mm was demonstrated by pure Metapex. The addition of a concentration of 1% ZnO provided a zone of inhibition of 6.67 ± 0.52 mm, and the addition of 3% ZnO further enhanced the antimicrobial action, resulting in an inhibition zone of 8.08 ± 0.58 mm. The mixture of 5% ZnO concentration with metapex showed the greatest antimicrobial efficacy with the largest inhibition zone of 11.42 ± 0.74 mm.

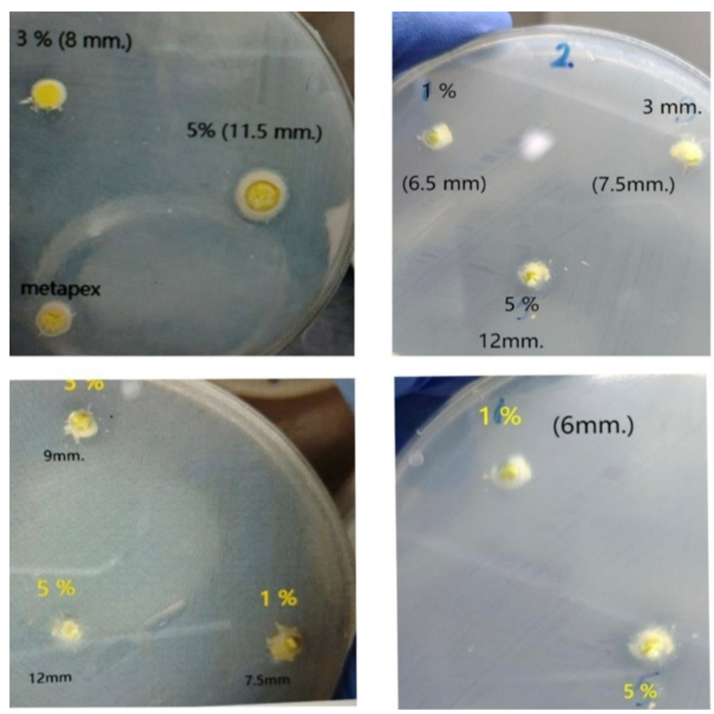

Figure 7. Agar diffusion test for testing the zone of inhibition of obturating materials against Enterococcus faecalis

Table 2. Antimicrobial Efficacy of Metapex with Varying Concentrations of Zinc Oxide Nanoparticles

Group	Zone of Inhibition (mm)							p-value*
	Mean	Std. Deviation	Std. Eror	95% Confidence Interval for Mean		Minimum	Maximum	
				Lower band	Upper bond			
Metapex	6.17	0.26	0.11	5.90	6.44	6.00	6.50	<0.001
Metapex + 1% ZnO	6.67	0.52	0.21	6.12	7.21	6.00	7.50	
Metapex + 3% ZnO	8.08	0.58	0.24	7.47	8.70	7.50	9.00	
Metapex + 5% ZnO	11.42	0.74	0.30	10.64	12.19	10.50	12.00	

*Kruskal-Wallis test

Pairwise comparisons were performed using the Mann-Whitney test, and the results are demonstrated in Table 3. Accordingly, the addition of ZnO nanoparticles at concentrations of 3% and 5% significantly increased the inhibition zone ($P<0.01$), indicating greater antibacterial activity. However, the addition of ZnO nanoparticles at a concentration of 1% did not significantly improve the inhibition zone compared to pure Metapex ($P=0.093$). Metapex + 5% ZnO showed the highest antibacterial activity, which was significantly greater compared to the other groups. These results are shown in chart form in Figure 8.

Table 3. Pairwise Comparison of Zone of Inhibition Between Study Groups

Group	Zone of Inhibition (mm)	
	p-value*	significance
Metapex Vs Metapex + 1% ZnO	0.093	Not significant
Metapex Vs Metapex + 3% ZnO	0.002	Significant
Metapex Vs Metapex + 5% ZnO	0.002	Significant
Metapex + 1% ZnO Vs Metapex + 3% ZnO	0.004	Significant
Metapex + 1% ZnO Vs Metapex + 5% ZnO	0.002	Significant
Metapex + 3% ZnO Vs Metapex + 5% ZnO	0.002	Significant

* Mann-Whitney Test

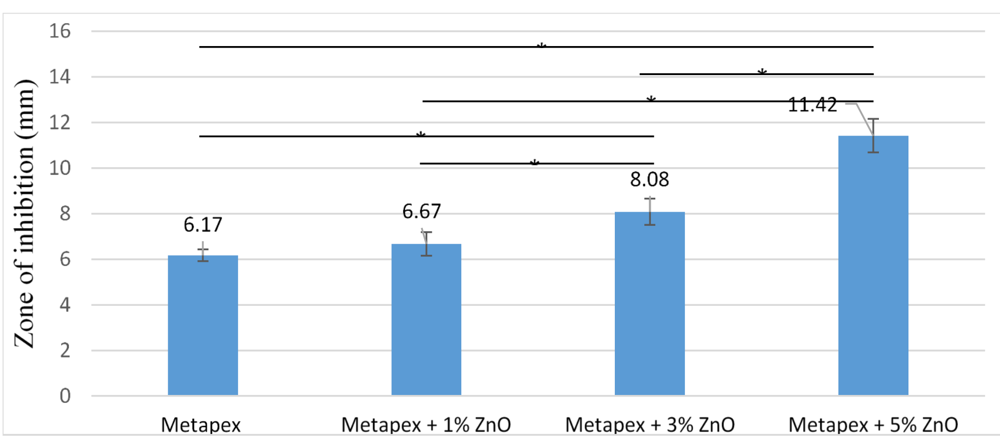

Figure 8. Comparison of the zone of inhibition between study groups

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Solubility test

The solubility of Metapex modified with varying ZnO nanoparticle concentrations is summarized in Table 4. Statistical analysis indicated notable differences among the groups (p = 0.003, ANOVA). Pure Metapex showed the highest solubility (35.17 ± 0.59), while increasing ZnO content inversely correlated with solubility: 32.93 ± 2.11 (1% ZnO), 27.77 ± 4.37 (3% ZnO), and 24.03 ± 1.97 (5% ZnO).

Table 4. Effect of Zinc Oxide Nanoparticle Concentration on the Solubility Profile of Metapex

Group	Solubility (%)							p-value*
	Mean	Std. Deviation	Std. Eror	95% Confidence Interval for Mean		Minimum	Maximum	
				Lower band	Upper bond			
Metapex	35.17	0.59	0.34	33.71	36.62	34.50	35.60	0.003
Metapex + 1% ZnO	32.93	2.11	1.22	27.70	38.17	30.60	34.70	
Metapex + 3% ZnO	27.77	4.37	2.52	16.92	38.61	22.80	31.00	
Metapex + 5% ZnO	24.03	1.97	1.14	19.13	28.93	22.70	26.30	

*ANOVA

Pair wise comparison was also conducted with Tukey's test and is given in Table 5. The incorporation of ZnO nanoparticles at concentrations of 3% and 5% was found to markedly reduce solubility (P<0.05). However, the addition of ZnO nanoparticles at a concentration of 1% did not significantly change solubility compared to pure Metapex (P=0.733). These results are shown in chart form in Figure 9.

Table 5. Pairwise Comparison of Solubility Between Study Groups

Group	Solubility (%)	
	p-value*	significance
Metapex Vs Metapex + 1% ZnO	0.733	Not significant
Metapex Vs Metapex + 3% ZnO	0.036	Significant
Metapex Vs Metapex + 5% ZnO	0.004	Significant
Metapex + 1% ZnO Vs Metapex + 3% ZnO	0.154	Not significant
Metapex + 1% ZnO Vs Metapex + 5% ZnO	0.014	Significant
Metapex + 3% ZnO Vs Metapex + 5% ZnO	0.367	Not significant

* Tukey HSDTest

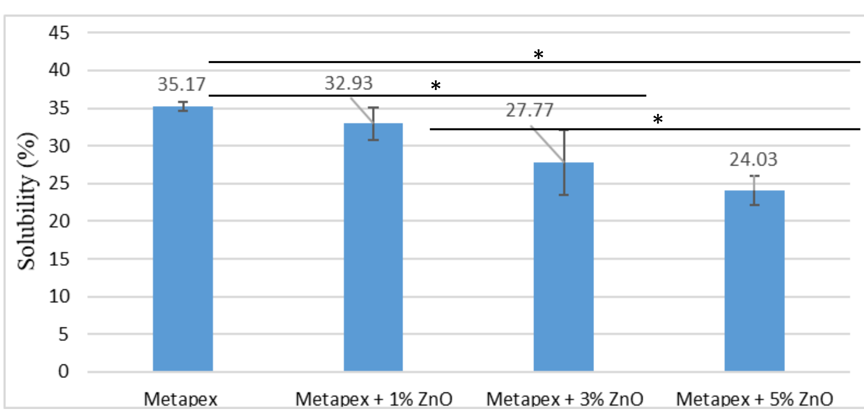

Figure 9. Pairwise comparison of solubility between study groups

DISCUSSION

Damage to primary teeth due to trauma or decay can compromise the maintenance of permanent tooth space. Therefore, pulpectomy is a mandatory procedure in cases of primary tooth damage, which involves removal of pulp tissue, removal of debris, filling, and obturation of the canal with appropriate materials.[13] Metapex is a biocompatible root canal sealer composed of calcium hydroxide and iodoform. The combined effect of these two substances induces healing.[14] It has been suggested that Metapex may be a more suitable choice for pulpectomy of primary teeth compared to other obturation materials on the market.[15] Root canal sealers should exhibit low solubility to ensure optimal obturation. High solubility may compromise the seal, leading to gap formation and endodontic treatment failure.[16] Thus, developing methods to minimize obturation material solubility may enhance therapeutic efficacy. For this reason, we chose to use Metapex in the current study. Since no prior research has been done, our goal was to improve its antibacterial characteristics and solubility in endodontic treatments by adding zinc oxide nanoparticles.

Nanotechnology has been introduced in endodontics to create materials that combine robust antibacterial and mechanical properties, resembling the characteristics of natural tissues.[17] ZnO NPs are among the latest nanomaterials introduced, known for their high safety and acceptable physicochemical properties. Moreover, ZnO NPs are considered dimensionally stable and highly compatible, with potent antibacterial properties.[18] In this regard, incorporating ZnO NPs into endodontic materials holds significant potential for enhancing their performance, which is why we are exploring their impact on improving the antibacterial and mechanical properties of Metapex in our study.

The study reveals a critical interplay between ZnO NPs concentration and the functional properties of Metapex. At lower concentrations (1% ZnO), the modified material did not show a significant increase in antimicrobial efficacy compared to pure Metapex. However, at higher concentrations of ZnO (3% and 5%), a significant enhancement in antibacterial activity was observed, with the largest inhibition zone achieved by the 5% ZnO concentration. This suggests a synergistic effect where ZnO NPs amplify antibacterial activity. Results of the present study demonstrate the effectiveness of ZnO NPs in enhancing the antibacterial properties of Metapex. Although the impact of adding ZnO NPs to Metapex has not yet been studied, the effect of adding these particles to certain dental materials has. The purpose of Jaber and Abbas's study was to examine the mechanical and bactericidal properties of ZnO nanoparticles added to glass ionomer (GI) restorations. ZnO nanoparticles have also been shown to drastically enhance the antibacterial activity of glass ionomer restorations based on the study findings.[19] Varghese et al. in their recent work examine the antibacterial activity of ZnO nanoparticles prepared with a green method. These findings established equal antibiotic activity against all oral pathogens including Candida albicans, Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, and against all the Lactobacillus species with differential sensitivity experienced between the different bacteria.[20]

The bactericidal mechanism of ZnO NPs involves several key processes. When ZnO NPs come into contact with bacterial cell walls, they lead to the production of free radicals. These free radicals, in turn, disrupt the integrity of the bacterial cell structure. Additionally, ZnO NPs release $Zn2+$ ions, which can further damage their internal processes. This interaction also leads to the generation of ROS, which contribute to oxidative stress within the bacterial cell, ultimately resulting in cell death.[21]

The present study found an inverse relationship between ZnO NPs content and material dissolution; Pure Metapex showed the highest solubility, which significantly decreased at 3% and 5% ZnO NPs concentrations. Similar reductions in solubility were reported by studies incorporating ZnO NPs into Grossman's sealer and CEM cement.[22,23] The higher concentration (10%) required for CEM compared to Metapex likely reflects intrinsic differences in their matrices and initial solubility levels. This reduction in solubility can be attributed to several factors: the formation of protective layers against moisture, enhanced chemical bonding, stabilization of the crystalline structure, and increased molecular density, all of which contribute to improved structural stability.[24,25]

It is important to acknowledge that this study was conducted in vitro. While it provides valuable insights into the antibacterial efficacy and solubility of ZnO NP-modified Metapex, the results must be interpreted within the context of inherent limitations. The controlled laboratory environment lacks

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the complex physiological conditions (e.g., dynamic fluid flow, pH variations, presence of organic molecules, host immune response) and the biological interactions encountered in vivo. Additionally, the antibacterial assessment was performed solely against Enterococcus faecalis; testing against a broader spectrum of oral pathogens and biofilms would strengthen the generalizability of the antimicrobial findings. While qualitative SEM analysis suggested homogeneity, quantitative assessment of nanoparticle dispersion and potential aggregation is necessary to better correlate uniformity with functional properties. Subsequent in vivo investigations are required to validate these results under clinical conditions.

Building upon these in vitro results, several critical avenues for future investigation are evident. The optimal choice of Metapex is to mix with 5% ZnO with superior antibacterial activity, largest inhibition zone, and largest root canal persistence to provide protection and natural resorption. While beyond this in vitro scope, the clinical translation of 5% ZnO-Metapex would require evaluation of handling properties during mixing and cost-benefit analysis relative to treatment efficacy.

CONCLUSION

The incorporation of ZnO nanoparticles (NPs) into Metapex elevates its antibacterial activity to the highest while diminishing its solubility. Among all concentrations tested, 5% ZnO NPs showed the greatest increase in antimicrobial activity with the largest zone of inhibition. Concurrent with this concentration was also the lowest solubility, optimally matching improved antibacterial activity with the lowest rate of dissolution. Hence, the addition of 5% ZnO NPs is a prospective alteration of pulpectomy treatments that optimally integrates both antibacterial activity and material stability.

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Conflict of Interest

The authors declare no conflicts of interest.

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