Original Article

Comparative Evaluation of the Antimicrobial Activity of NeoPutty MTA and Modified NeoPutty MTA: An *In Vitro* Study

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ABSTRACT

Aim: Mineral trioxide aggregate (MTA) is a relatively new versatile dental material. MTA has many advantages as well as disadvantages. To reduce most of the drawbacks of MTA, a premixed bioceramic MTA, NeoPutty MTA, was introduced in 2020. In this study, we assessed the antimicrobial activity of the newer MTA, NeoPutty MTA. We modified NeoPutty MTA and compared both against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. **Materials and Methods:** Using the agar diffusion method, NeoPutty MTA was tested for antibacterial activity against the above-mentioned microorganisms. A base layer of Petri plates was done using Muller–Hinton agar for *S. aureus*, *E. coli*, and *P. aeruginosa* and brain heart infusion agar for *E. faecalis*. A total of 32 plates were employed; the plates were divided randomly into four test groups having eight plates each, so microorganisms were tested eight times. Three cavities were made in agar and filled with freshly mixed materials after 24 h. A pour plate seeded the microorganisms. The plates were pre-incubated for 2 h at room temperature and incubated at 37°C for 24 h. An independent observer measured the inhibition zone diameters. **Results:** NeoPutty MTA, when tested alone, did not show much antibacterial activity against *E. faecalis*, *S. aureus*, and *E. coli* but had significant antimicrobial activity against *P. aeruginosa* when used at different concentrations. Modified NeoPutty (NeoPutty with antibiotics added individually) showed significant antibacterial activity against these microorganisms, as seen by the zone of inhibition of these bacteria. **Conclusion:** Modified NeoPutty with antibiotics has a better antimicrobial effect than NeoPutty MTA.

Keywords: *Antibiotics, antimicrobial, Enterococcus faecalis, Escherichia coli, NeoPutty MTA, Pseudomonas aeruginosa, Staphylococcus aureus*

Introduction

Mineral trioxide aggregate (MTA) has been one of the most studied materials in the past 20 years. MTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, calcium sulfate, and bismuth oxide.[1] MTA has many preferable properties in terms of its biocompatible nature: bioactive, hydrophilic, radiopaque, good sealing ability, and low solubility. The most important advantages of dentistry are its biocompatible nature and sealability.[2] High biocompatibility ensures optimal healing responses. This has been studied histologically with the formation of new cementum in the periradicular tissue area and a weak inflammatory response with bridge formation in the pulp area.[3] The

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seal achieved is due to its expansion and contraction properties being very similar to dentin, resulting in high resistance to marginal leakage.[4] MTA materials are derived from a Portland cement parent compound. Although these compounds are similar in some respects, Portland cement and MTA are different. MTA, used in dentistry, has to undergo further processing and purification to lower its molecular size and toxicity. MTA was first introduced to the dental specialty in 1993 while receiving Food and Drug Administration approval in 1998.[5] In 1999, ProRoot MTA (Dentsply Tulsa Dental Specialties, Johnson City, Tennessee) was the first commercially available MTA product to be launched in the USA. MTA is available in both gray and white colors. The first MTA products were gray, and most of the previous research was done on this gray MTA. Due to the discoloration that was reported when gray MTA was used, the white version of MTA was introduced for commercial use in 2002.[6] The difference between the two colors is mainly due to a decrease in the concentrations of iron, aluminum, and magnesium oxides in white MTA. Next, MTA Plus was introduced by Avalon Biomed (Houston, Texas). The specific surface area of MTA Plus is 1.537 m^2/g, which is larger than the other MTAs' values.[7] The larger specific surface area provided more surface for the cement reaction, resulting in a faster reaction. NeoPutty MTA was launched in 2020 by NuSmile (Houston, Texas) to minimize most of MTA's drawbacks. This material was said to be premixed, bioceramic, nonstaining, resin-free, and it does not dry out when used. NeoPutty MTA has been shown to have similar physical and mechanical properties to traditional MTA materials, including good sealing ability, biocompatibility, and radiopacity. It has also been shown to have a shorter setting time than traditional MTA materials, which can reduce treatment time and improve patient comfort.[8] This new material has antibacterial properties because of the alkaline pH of the material. Ideal endodontic cement should have bacteriostatic or bactericidal properties. MTA has the drawback of minimal antibacterial effect, especially against *Enterococcus faecalis*, which is the major microorganism responsible for the progression of pulpal and periradicular diseases and endodontic failures. *E. faecalis* forms a minor part of the microbial flora in uninstrumented canals, while it is a main etiologic factor for periradicular lesions that develop following the endodontic treatment. In various studies, adding certain additives, such as metallic silver, chlorhexidine, and calcium fluoride, has reduced the antibacterial counts.[9,10]

The topical use of antibiotics has been done in endodontics for pulp capping, as an intracanal medicament in root canal treatments, regenerative endodontics, and tooth avulsion cases for better healing. Adding antibiotics to NeoPutty MTA may enhance MTA's antibacterial activity, which still needs to be improved, even in the newer versions of MTA. NeoPutty MTA is a newer material that claims to have overcome almost all the disadvantages present in MTA but still its antibacterial property has not been tested. Here, we would check the antibacterial property of NeoPutty MTA and compare the result with the addition of antibacterials (antibiotics) with NeoPutty MTA. The rationale is to enhance the properties of this new material and make it better for clinical usage.[11]

The present study was aimed to evaluate the antibacterial properties of NeoPutty MTA and NeoPutty MTA with antibiotics (NeoPutty + clindamycin, NeoPutty + ciprofloxacin, and NeoPutty + metronidazole) against *E. faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The null hypothesis is that the proposed modified MTA is superior to NeoPutty MTA

Materials and Methods

Agar diffusion test

The *in vitro* study was conducted on double-layered plates, in which the base layer was made of 10 mL of sterilized Muller–Hinton (MH) agar poured in 2 × 10 cm sterilized Petri plates. Three uniform cavities (4 mm diameter, one for each test material) were punched at equidistant points in agar utilizing a sterile copper coil after 24 h [Figure 1]. The cavities were filled immediately by materials after being mixed according to the manufacturer's instructions. Antibacterial activities of the selected material, NeoPutty MTA,

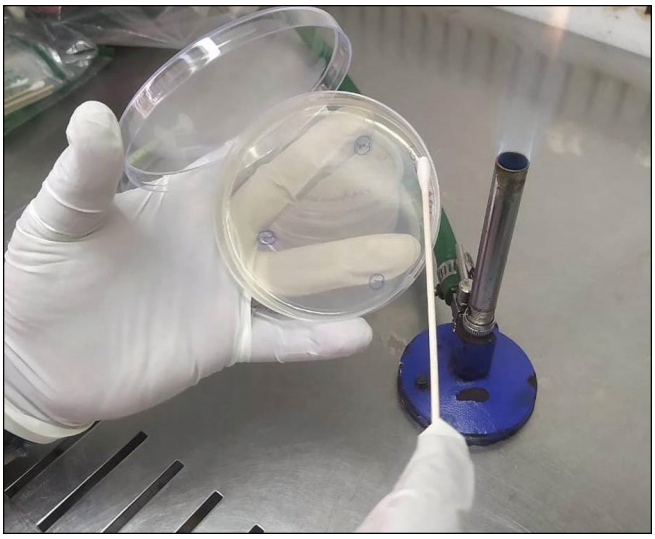

Figure 1: Inoculation of bacteria

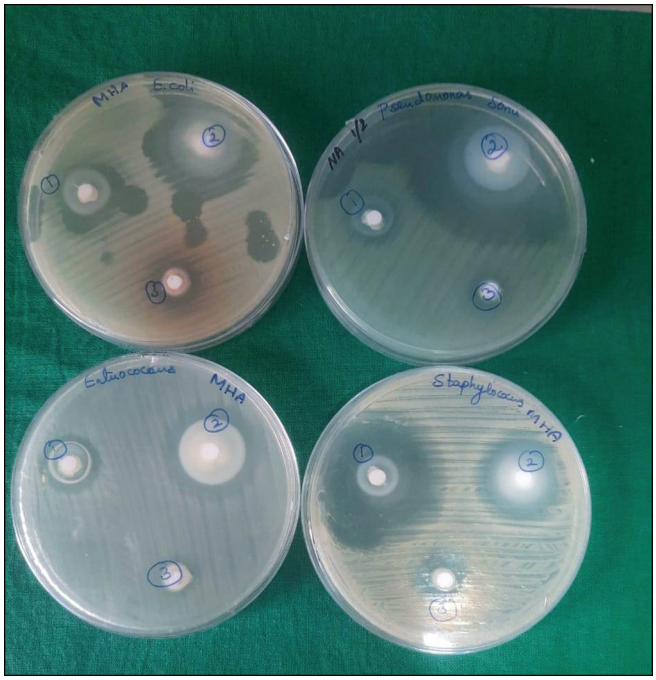

Figure 2: Zones of inhibition of different bacteria with modified NeoPutty MTA

were evaluated against the *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *E. coli* using an agar diffusion method. After activation from the stock culture of clinical strains, microorganisms were maintained in MH broth until used. Overnight cultures of the microorganisms were used. All the microbial strains were grown at 37°C for 24 h in MH broth and then seeded into 15 mL of the MH agar to produce a turbidity of 0.5 on the McFarland scale, corresponding to a concentration of 10^8 colony forming unit per milliliter. This broth was used as the second layer. The seeded agar was added over the plates immediately after the insertion of freshly mixed test materials. The plates were kept at room temperature for 2 h for prediffusion of the materials and then incubated at 37°C for 24 h [Figure 2]. Positive and negative controls were prepared, maintaining the plates with and without inoculums, for the same period and under identical incubation conditions. All assays were carried out under aseptic conditions.

Antibacterial activity

At first, we tested the antibacterial activity of NeoPutty MTA against the microorganisms mentioned above, namely *S. aureus*, *E. coli*, *P. aeruginosa*, and *E. faecalis*. A total of 32 plates were employed; the plates were divided randomly into four test groups having eight plates each, so microorganisms were tested eight times.

NeoPutty MTA was prepared in varying concentrations, i.e., 5, 25, and 1.25 mg/mL, by dissolving the compound in sterile distilled water. Fresh cultures of the test organisms were prepared, and the turbidity was adjusted to 0.5 McFarland standards. Lawn cultures of the test organisms were made onto the MH agar for *S. aureus*, *E. coli*, and *P. aeruginosa* and onto the sterile brain heart infusion agar for *E. faecalis*. Wells were cut onto the surface of the agar, and the extracts at varying concentrations were added to appropriate wells. All the plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured using an antibiotic zone measuring scale, and the results were recorded in millimeter.

We tested the NeoPutty MTA mixed with antibiotics individually (modified NeoPutty MTA). Although triple antibiotic paste is used more often in endodontics, we preferred to try this new material with a particular antibiotic as we wanted to know the inhibitory effect of individual antibiotics before testing the triple antibiotic paste. The other reason for testing individual antibiotics with NeoPutty MTA was the difficulty of mixing NeoPutty MTA with triple antibiotic paste as the mixture was inconsistent. The testing of triple antibiotic paste mixed with NeoPutty MTA would be done in further studies along with various physical properties of modified NeoPutty MTA in further *in vitro* tests.

Neoputty MTA was mixed with antibiotics (clindamycin 300mg, ciprofloxacin 500mg, and metronidazole 400mg), one by one and tested. NeoPutty was mixed with a 1 mg/mL concentrations of the antibiotics. Fresh cultures of the test organisms were prepared, and the turbidity was adjusted to 0.5 McFarland standards. Lawn cultures of the test organisms were made on to the MH agar for *S. aureus*, *E. coli*, and *P. aeruginosa* and onto the sterile brain heart infusion agar for *E. faecalis*. Wells were cut onto the surface of the agar, and 50 µL of the prepared compounds were added to appropriate wells. All the plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured using an antibiotic zone measuring scale, and the results were recorded in millimeter by an independent observer.

Statistical analysis was done with software (IBM SPSS Statistics, Version 20.0; IBM Corp., Armonk, New York) and the results thus obtained were evaluated.

Results

The positive control showed the growth of bacteria, but the negative controls did not show growth. All bacterial strains had inhibition with the test material (NeoPutty combined with antibiotics), but only *P. aeruginosa* showed inhibition with NeoPutty MTA. The antimicrobial activities of test materials (NeoPutty MTA and modified NeoPutty MTA) determined by

Table 1: NeoPutty MTA at various concentrations

Concentration of NeoPutty MTA (mg/mL)	Zone of inhibition			
	Enterococcus faecalis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
5	—	12 mm	16 mm (SD ± 1.4 mm)	8 mm
25	—	—	15 mm (SD ± 0.8)	—
1.25	—	—	13 mm (SD ± 0.4)	—

— = no activity, number of assays = three each with the mean being noted here

Table 2: The antibacterial activity of different antibiotics with NeoPutty MTA against four bacterial species

Concentration of NeoPutty MTA (mg/mL)	*Enterococcus faecalis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
NeoPutty MTA with clindamycin 300 mg	18 mm (SD 18 ± 1.4)	35 mm (SD 35 ± 2.3)	12 mm (SD 12 ± 0.8)	23 mm (SD 23 ± 1.2)
NeoPutty MTA with ciprofloxacin 500 mg	27 mm (SD 27 ± 1.9)	25 mm (SD 25 ± 1.7)	42 mm (SD 42 ± 2.7)	35 mm (SD 35 ± 1.9)
NeoPutty MTA with metronidazole 400 mg	0	17 mm (SD 17 ± 3.1)	10 mm (SD 10 ± 1.5)	15 mm (SD 15 ± 1.1)

SD = standard deviation

the means and standard deviation of growth inhibition zones in millimeters on all test microorganisms after 24 h are given in Tables 1 and 2.

The analysis of variance (ANOVA) test for intergroup variation for *P. aeruginosa* showed an F value of 70.90, which was statistically significant ($P < 0.01$). Intergroup comparison using ANOVA, the F value was 2390.08 for the *E. faecalis* group, 8343.20 for *S. aureus*, 6270.16 for *P. aeruginosa*, and 2097.14 for *E. coli*. All the antibiotics showed significant results against all four bacterial species ($P < 0.01$). The results showed significantly better results when NeoPutty MTA was added with different antibiotics compared to NeoPutty MTA used alone, which had significantly better results on the inhibition of *P. aeruginosa*.

Discussion

Role of micro-organisms in endodontic infections

The most notable reason for endodontic failure is the presence of some species of bacteria inside the root canal system. Those bacteria are more resistant to disinfection agents, causing a persistent intra- or extraradicular infection. *E. faecalis* is resistant to high pH and has the ability to invade the dentinal tubules. Thus, it is highly resistant to intracanal medicaments.[12] *S. aureus* is one of the important resistant microorganisms frequently isolated from recurrent root canal treatments. It plays a major role in the etiology of primary endodontic infections and persistent infections among break sessions during root canal therapy when the root canal was left open.[12] *E. coli*, a Gram-negative facultative anaerobe, constitutes the biofilm that recovers the wall of the root conduits with dead pulp. *E. coli* is not a common bacterium found in dental pulpal infections.[13] Gram-positive bacteria, such as *Streptococcus* species and *S. aureus*, typically cause dental pulpal infections. In dental infections, *S. aureus* is often associated with abscesses and cellulitis. It is one of the most common bacteria found in dental infections, along with *Streptococcus* species and anaerobic bacteria.[14-16] *P. aeruginosa* is known for its ability to form biofilms, which are complex communities of bacteria that adhere to surfaces, such as teeth or implants. These biofilms can be difficult to eradicate with conventional antibiotics, making treating of infections with *P. aeruginosa* quite challenging.[15] Treatment of *P. aeruginosa* infections in dentistry may involve using antibiotics, such as ciprofloxacin or ceftazidime, which are known to be effective against this bacterium.

Topical antibiotics can be used in pulp therapy in dentistry as an adjunct to conventional treatment approaches, such as pulpotomy or pulpectomy. Topical antibiotics are used to control the infection in the pulp and periapical tissues and to promote healing.[17-19] While topical antibiotics can effectively control infection in the pulp and periapical tissues, their use should be limited to specific cases where the infection is severe or where conventional treatment approaches have failed. Overuse of antibiotics can lead to antibiotic resistance, making treatment of infections more difficult in the future.[20] Therefore, it is essential to use topical antibiotics judiciously and only in cases where they are indicated. Here, in this study, we used antibacterials individually in order to avoid antibiotic resistance that can develop.

MTA has been shown to possess antibacterial activity against various microorganisms commonly found in dental infections, including *E. faecalis*, *Streptococcus mutans*, and *S. aureus*.[21]

Antibacterial activity of MTA

The antibacterial activity of MTA is believed to be due to its alkaline pH, which creates an unfavorable environment for bacterial growth. Additionally, MTA contains calcium and silicate ions, which can stimulate hydroxyapatite formation and promote mineralization in the surrounding tissues, further inhibiting bacterial growth. Several studies have demonstrated the antibacterial activity of MTA *in vitro*, with MTA showing a significant reduction in bacterial growth compared to controls.[22-24] *In vivo* studies have also demonstrated that MTA can reduce bacterial colonization and promote healing in periapical lesions.[25] However, it is important to note that MTA's antibacterial properties may be limited in certain circumstances. For example, MTA may be less effective against biofilm-forming bacteria or in the presence of organic matter or blood. In these cases, additional antimicrobial agents may be necessary to eliminate the infection entirely. Several studies have investigated the addition of various antimicrobial agents to MTA to enhance the antibacterial properties of MTA. Some of the commonly studied additives include:

1. Chlorhexidine: Chlorhexidine is a broad-spectrum antimicrobial agent commonly used in dentistry. Studies have shown that adding chlorhexidine to MTA can significantly enhance its antibacterial properties, particularly against *E. faecalis*. The study had similar results as our study with the addition of chlorhexidine against *E. faecalis*.[26]
2. Silver nanoparticles (AgNPs): AgNPs have been shown to have potent antimicrobial activity against a wide range of microorganisms, including bacteria, viruses, and fungi. Studies have demonstrated that adding AgNPs to MTA can significantly enhance antibacterial properties. When mixed with AgNPs, MTA enhanced antibacterial activity against anaerobic endodontic-periodontal pathogens, e.g., *E. faecalis* and *P. aeruginosa*, and improved antifungal activity against *C. albicans*. In our study, we got better antibacterial activity with the addition of antibiotics against *E. faecalis* and *P. aeruginosa*.[27]
3. Propolis: Propolis is a resinous substance collected by bees that has been shown to have antimicrobial properties. Studies have demonstrated that adding propolis to MTA can significantly enhance its antibacterial properties against *E. faecalis* and *S. aureus*. The addition of propolis has a similar antibacterial effect as a triple antibiotic paste. In our study too, we got similar results with *E. faecalis* and *P. aeruginosa* despite using single antibiotic agents with NeoPutty MTA.[28]
4. Fluoride: Several studies have reported on the antimicrobial effects of fluoride-containing MTA. For example, one study found that adding sodium fluoride to MTA improved its antibacterial activity against *S. mutans*, the bacteria that can cause dental caries. Another study showed that MTA mixed with calcium fluoride had better antibacterial effects against *E. faecalis*, the bacteria commonly associated with failed endodontic treatments, compared to MTA alone. Our study showed similar results with modified NeoPutty MTA as modified NeoPutty inhibited *E. faecalis*, *P. aeruginosa*, *and S. aureus*.[10] For testing the antibacterial effect of NeoPutty MTA and modified Neoputty MTA, four bacteria were used, namely *E. faecalis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. Previous studies have reported that MTA failed to inhibit the growth of *E. faecalis*.[29] In our study, we have found that addition of clindamycin and ciprofloxacin with NeoPutty MTA has restricted *E. faecalis*.

NeoPutty MTA is a premixed, ready-to-use, bioceramic material used in endodontic procedures. It is designed to overcome some drawbacks of traditional MTA materials, such as the difficulty in handling and mixing the powder and liquid components and the long setting time. NeoPutty MTA is a relatively new material in dentistry, and limited studies are available on its use. However, some studies have been conducted to evaluate its physical and mechanical properties and clinical performance. One recent study compared NeoPutty MTA's properties with those of ProRoot MTA and Biodentine, two other commonly used dental materials. The study found that NeoPutty MTA had similar or better physical and mechanical properties than the other materials, including higher compressive strength, better setting time, and lower solubility.[30]

Another recently published study evaluated the clinical performance of NeoPutty MTA in pulpotomy procedures in primary molars. The study found that NeoPutty MTA had a success rate of 91.7% at 6-month follow-up and was effective in achieving pulpal healing and maintaining the vitality of the treated teeth.[31]

This study individually tested the antibiotics, clindamycin, ciprofloxacin, and metronidazole and got good results against the common microorganisms encountered in the root canals of primary and permanent teeth.

Strengths and weakness of the study

There has yet to be a study on the antibacterial effect of NeoPutty MTA. We tested and found this material to be having poor antibacterial activity. The results stated that adding various antibiotics in NeoPutty MTA can inhibit four major bacteria considered to be present across multiple pulp pathologies. The addition of antibiotics can enhance its antibacterial properties and thus can be an advantage in pulpal therapy in primary and permanent teeth. The study suggests that NeoPutty MTA mixed with antibiotics may be a promising material for use in various dental procedures. Still, more research is needed to evaluate its safety and effectiveness fully. The addition of antibiotics can affect the setting time and strength of the material (NeoPutty MTA). The physical properties that are affected and the clinical applicability of this modified material need further testing, which will be subsequently done in further studies.

In vitro antimicrobial susceptibility testing hasits demerits; thus, correlating *in vitro* results with the *in vivo* activity is difficult. Further research is warranted on the physical properties, e.g., setting and working times after the addition of antimicrobials to the NeoPutty MTA, as well as on testing the antimicrobial effect of already set NeoPutty MTA samples, as the results could be influenced by the dissolution and diffusibility of the test agent through the agar medium.

Conclusion

Mineral trioxide aggregate (MTA) has demonstrated notable antibacterial activity, contributing to its effectiveness in various endodontic applications. The material's inherent properties, such as its high pH and ability to release calcium hydroxide, contribute to its antibacterial effects.The newer MTA,NeoPutty MTA has antibacterial properties but it was shown in this study, to be enhanced with addition of antibacterials which will make NeoPutty MTA a more potent endodontic material.

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Conflicts of interest

We declare that we have no competing interest.

Authors contributions

SA conceived the idea, collected and analyzed the data, and wrote the manuscript. DG made the analysis and proof read the manuscript. DP analyzed the results. BS contributed data and analysis tools. AS provided the resources. SA contributed in project administration. All authors have approved the final manuscript.

Ethical policy and Institutional Review board statement

Ethical permission vide letter number is IEHC/SDC/PEDO/2204/22/07.

Patient declaration of consent

In vitro study permission to reproduce material from other sources: none.

Data availability statement

Data can be made available on request.

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