

## Comparative evaluation of antimicrobial activity of three cements: new endodontic cement (NEC), mineral trioxide aggregate (MTA) and Portland

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**Abstract:** Using the agar diffusion method, we conducted an *in vitro* study to evaluate the antimicrobial activity of mineral trioxide aggregate (MTA), new endodontic cement (NEC) and Portland cement at different concentrations against five different microorganisms. A base layer was made using Muller-Hinton agar for *Escherichia coli* (ATCC 10538) and *Candida* (ATCC 10231). For *Actinomyces viscosus* (ATCC 15987), *Enterococcus faecalis* (ATCC 10541) and *Streptococcus mutans* (ATCC 25175) blood agar medium was used. Wells were formed by removing the agar, and the materials were placed in the well immediately after manipulation. The plates were kept at room temperature for 2 h for prediffusion, and then incubated at 37°C for 72 h. The inhibition zones were then measured. The data were analyzed using ANOVA and the Tukey test to compare the differences among the three cements at different concentrations. The positive controls showed bacterial growth, while the negative controls showed no bacterial growth. All materials showed antimicrobial activity against the tested strains except for *Enterococcus faecalis*. NEC created larger inhibition zones than MTA and Portland cement. This difference was significant for Portland

cement ( $P < 0.05$ ), but not for MTA ( $P > 0.05$ ). Among the examined microorganisms, the largest inhibition zone was observed for *Actinomyces* group ( $P < 0.05$ ). The antimicrobial activity of the materials increased with time and concentration ( $P < 0.05$ ). It was concluded that NEC is a potent inhibitor of microorganism growth. (J Oral Sci 51, 437-442, 2009)

Keywords: antimicrobial activity; new endodontic cement; mineral trioxide aggregate; Portland cement.

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### Introduction

Microorganisms play a key role in the development and progression of pulpal and periapical disease as well as in endodontic treatment failure (1). Failure of initial endodontic treatment or accidental mishaps, such as perforations, can be successfully treated either nonsurgically or surgically. Treatment outcome will depend on successful elimination of the associated microorganisms and infected tissues as well as effective sealing of the root-end or perforation site to prevent future recontamination (2).

Several independent studies have shown that certain microorganisms are repeatedly recovered from previously root-filled teeth that have become infected. These are chiefly *Enterococcus*, *Actinomyces*, *Propionibacterium*, yeasts and *Streptococcus*, with occasional reports of other types (3).

When nonsurgical endodontic therapy is unsuccessful or not possible, endodontic surgery is needed for tooth

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salvage. This procedure involves exposure of the involved apex, root resection, preparation of a class I cavity at the resected root-end, and insertion of a root-end filling material in the prepared cavity. The aim of placing a root-end filling material is to develop an apical seal at the end of the resected root (4). An ideal root-end filling material should produce a complete apical seal, be nontoxic, well tolerated by the periradicular tissues, non-resorbable, dimensionally stable, easy to manipulate, and radiopaque (5). In addition, it should be bactericidal or bacteriostatic.

Although numerous materials have been recommended as root-end filling materials, none has so far been found to be totally ideal. Most endodontic failures are attributable to inadequate cleansing of the root canal and egress of bacteria and other antigens into the periradicular tissues (4). Therefore, in addition to sealing ability and biocompatibility, root-end filling materials should ideally have some antibacterial activity to prevent bacterial growth (2).

ProRoot mineral trioxide aggregate (MTA) is marketed as gray- and white-colored preparations, both of which are composed of 75% Portland cement clinker, 20% Bismuth oxide and 5% gypsum by weight. MTA is a powder that consists of fine hydrophilic particles that, in the presence of water or moisture, forms a colloidal gel that solidifies to form hard cement within approximately 4 hours. The more esthetic white-color preparation lacks tetra calcium aluminoferrite (6).

Recently, new endodontic cement (NEC) consisting of different calcium compounds (e.g., calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide and calcium chloride) has been developed. Its physical properties conform to ISO 6876:2001. The clinical applications of NEC are similar to those of MTA, and both cements have a similar working time, pH and dimensional stability (7). In a previous study, Asgary et al. (8) found that NEC had significantly more pronounced antibacterial properties than MTA. However, no reported studies have evaluated the antifungal activity of NEC.

The purpose of this study was to investigate and compare the antibacterial and antifungal effects of NEC, MTA and Portland cement on some selected oral microorganisms.

## Material and Methods

The test materials – MTA (Dentsply, Tulsa dental, OK, USA), NEC (Shahid Beheshti University, Tehran, Iran) and Portland cement (Ciman Shargh, Mashhad, Iran) – were manipulated strictly in accordance with the manufacturer's instructions. The antimicrobial activity of the endodontic cements at five different concentrations was evaluated by

the agar diffusion method against five reference strains: *Enterococcus faecalis* (ATCC 29212) *Escherichia coli* (ATCC 33780), *Streptococcus mutans* (ATCC25175), *Candida* (ATCC 10231) and *Actinomyces viscosus* (15987).

Each endodontic cement was evaluated at concentrations suggested by the manufacturer: powder/liquid = 3/1 and 1/2, 1/4, 1/8 and 1/16. Bacteria were diluted to obtain a suspension of approximately  $5 \times 10^8$  colony-forming units/ml (0.5 in a McFarland nephelometer) in sterile TSB (Trypticase Soy Broth) (Merck, Germany). Microbial strains were confirmed by both Gram staining and colony-forming and growth characteristics. *Enterococcus faecalis* and *Candida* suspensions were inoculated with sterile cotton swabs onto Muller-Hinton agar plates (Merck) and the other strains were inoculated onto blood agar media (Merck).

Wells 4 mm in diameter and 4 mm deep were prepared on plates with a copper puncher, and immediately filled with freshly manipulated test materials. Positive and negative controls consisted of plates incubated for the same period under identical conditions with and without inocula.

After prediffusion of the test materials for 2 h at room temperature, all the plates were incubated at 37°C and evaluated at 24, 48 and 72 h. Microbial inhibition zones were measured with a 0.5-mm precision ruler (Figs. 1-3) and the results were expressed as the mean and standard deviation of three independent experiments. Data were analyzed statistically by ANOVA and the Tukey test to

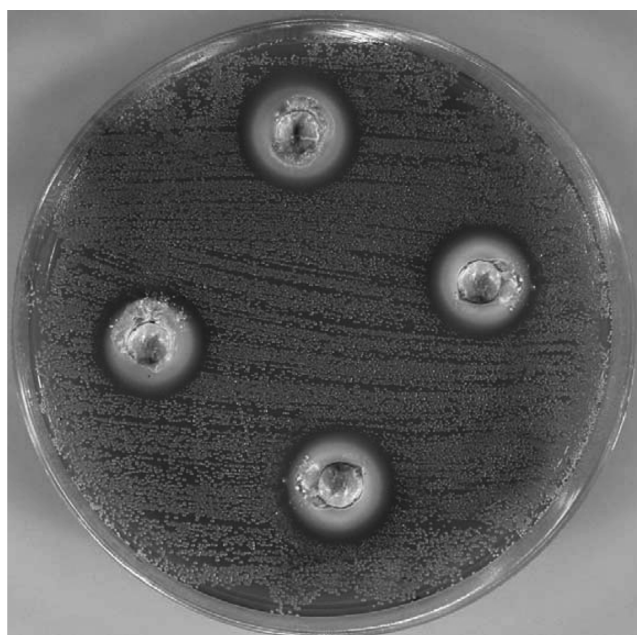


Fig. 1 Inhibition zone against *Candida* with NEC.

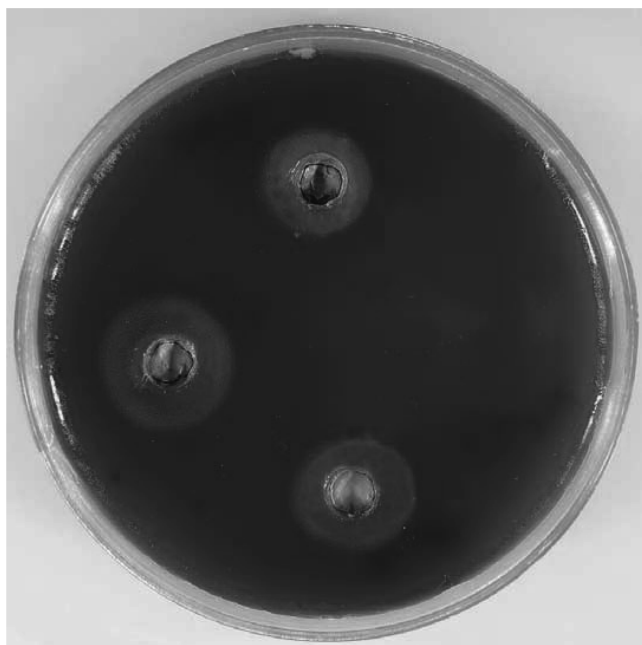


Fig. 2 Inhibition zone against Actinomyces with MTA.

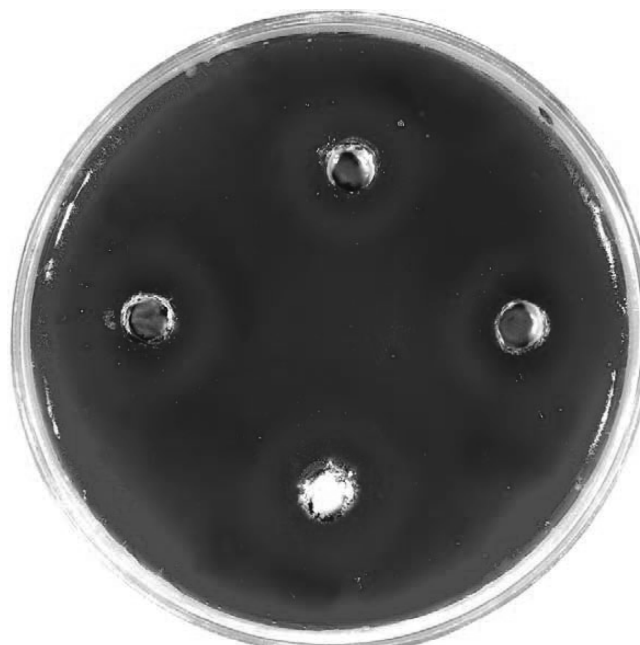


Fig. 3 Inhibition zone against Actinomyces with NEC.

Table 1 Mean and standard deviation of diameters of inhibition zones for MTA, NEC and Portland

Materials	Mean (Diameter)	SD
Portland	4.7	7.8
MTA	6.9	8.4
NEC	8.3	9.5
Total	6.6	8.7

$P < 0.05$ ,  $F = 12.018$

Table 2 Mean and standard deviation of diameters of inhibition zones for different microorganisms

Microorganisms	Mean	SD
<i>E. faecalis</i>	0	0
<i>S. mutans</i>	10.6	8.5
<i>E. coli</i>	5.0	6.2
<i>Candida</i>	5.8	6.7
<i>Actinomyces</i>	16.3	10.8
Total	8.6	9.0

$P < 0.05$ ,  $F = 62.657$

compare the differences among MTA, NEC and Portland cement at different concentrations.

## Results

The antimicrobial activities of MTA, NEC and Portland cement are shown in Table 1. The positive control showed bacterial growth, while the negative control showed no growth.

The antimicrobial action of NEC on all the microorganisms tested was superior to that of MTA and Portland, showing a mean inhibition zone of 4.7 mm. This difference was significant for Portland cement ( $P < 0.05$ ), but not for MTA ( $P > 0.05$ ). Also, the difference between MTA and Portland cement was significant, and MTA showed more antimicrobial activity ( $P > 0.05$ ).

All cements were significantly more effective against

*S. mutans* than against *Candida* and *E. coli* ( $P < 0.05$ ). Nevertheless, all the cements were incapable of inhibiting the growth of *E. faecalis*. All the cements were more effective against *Candida* than against *E. coli*, but not to a significant degree ( $P > 0.05$ ). For all three cements, the diameters of the inhibition zones for *Actinomyces* were significantly larger than for *S. mutans*, *Candida*, and *E. coli* ( $P < 0.05$ ) (Table 2).

A decrease in the cement concentration resulted in a decrease in the inhibition zone ( $P < 0.05$ ). No significant difference in effect was found between the initial and 1/2 concentrations ( $P > 0.05$ ), and also between the 1/2 and 1/4 concentrations ( $P > 0.05$ ) (Table 3).

The data demonstrated a significant increase in the

Table3 Mean and standard deviation of diameters of inhibition zones for different concentrations

Concentration	Mean	SD
Initial	13.4	10.2
1/2	11.4	9.3
1/4	10.0	8.1
1/8	6.8	8.0
1/16	2.7	5.4
Total	8.9	9.2

$P < 0.05, F = 29.114$

Table4 Mean and standard deviation of diameters of inhibition zones for different period

Time	Mean	SD
24 h	3.2	5.5
48 h	5.1	7.0
72 h	7.9	9.2
Total	6.6	8.7

$P < 0.05, F = 49.758$

inhibition zone with time, except between 24 and 48 h ( $P > 0.05$ ) (Table 4).

## Discussion

In this study, we investigated the antimicrobial activity of MTA, NEC, and Portland cement. The microorganisms utilized included facultative bacteria and yeast, which are predominant in persistent or refractory periapical lesions of teeth subjected to periapical surgery. *E. faecalis* and *Actinomyces* are robust microorganisms that may infect root canals (9,10) and are more likely to be found in cases of failed endodontic therapy than in cases of primary infection (11). *E. coli* is sometimes recovered from root canals and represents a standard organism used in antimicrobial testing (12,13). *C. albicans* has the ability to form biofilms on different surfaces, and may be involved in cases of persistent and secondary infection (14). *S. mutans* may have a major influence on both the initial pulpal lesion and subsequent pulpal pathology (15).

It should be noted that in this study, we used different concentrations of cements to establish the minimum inhibitory concentrations for MTA, NEC and Portland cement against the tested microorganisms. We employed the agar diffusion method, which is the most commonly employed technique for evaluation of antimicrobial activity (5). This technique has been used by many authors in

antimicrobial studies (16-18), but differences in agar medium, diffusion capacity of inhibitory agents, bacterial strains and cellular density may interfere with the formation of inhibition zones around materials used in antimicrobial testing (19,20).

Our results showed that NEC had higher antimicrobial activity than MTA, but not to a significant degree, and the antibacterial activities of MTA and NEC were significantly higher than that of Portland cement. This result suggests that NEC contains more potent antibacterial inhibitors than MTA. Alkaline earth metal oxide and hydroxides (e.g. calcium oxide and calcium hydroxide, calcium phosphate, and calcium silicate) are important constituents of NEC. When NEC is transferred to agar plates and makes contact with medium,  $\text{Ca(OH)}_2$  dissociates into calcium and hydroxyl ions, increasing the pH and calcium concentration. These mechanisms may partly explain the more favorable antibacterial activity of this material. An alternative explanation is that the antimicrobial components of NEC have better diffusion properties than those of MTA and Portland cement (8). The antimicrobial effect of MTA against the test organisms could be attributable to its high pH or release of diffusible substance(s) into the growth medium (21), as demonstrated by Duarte et al. (22).

Torabinejad et al. (5) observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. It is known that pH levels in the order of 12 can inhibit most microorganisms (23). Asgary et al. (8) found that NEC had a significantly more pronounced antibacterial effect than MTA.

No previous studies have evaluated the antimicrobial activity of MTA, NEC, and Portland cement against *Actinomyces*. The present study revealed that the diameter of the inhibition zone varied significantly according to the microorganism tested. For all three cements, the largest inhibition zones formed around *Actinomyces*. Therefore, our results suggested that *Actinomyces* is not resistant to these antimicrobial materials if exposed directly to them. Fimbriae on the surface of *Actinomyces* may contribute to its pathogenicity (24,25).

All of the tested cements had more pronounced antimicrobial effects against *S. mutans* than against *E. coli*, and *Candida*. However, all three were ineffective against *E. faecalis*. The resilient characteristics of *E. faecalis* in endodontic infections are well documented (1,26,27). Estrela et al. (19) demonstrated that MTA had no antimicrobial activity against *E. faecalis*, and Torabinejad et al. (5) detected no efficacy against *E. faecalis*, similarly to our findings.

It has been reported that *E. faecalis* shows resistance to intra-canal dressings containing calcium hydroxide (28,29). In this regard, it has been reported that the main chemical

component released by MTA in aqueous solution is calcium hydroxide (30). Our results agree with those of Tanomaru et al. (31), who reported that MTA and Portland cement were effective against *E. coli*, and also those of Asgary et al. (8), for NEC and MTA. However, our results differ from those of Torabinejad (5) and Estrela (19) in that they did not find any inhibitory effect of MTA against *E. coli*. The disagreement between their results and ours could be attributable to the available nutrients, level of oxygen tension, incubation period, methods of evaluation, and different laboratory set-ups employed.

Many studies have demonstrated antifungal activity of MTA and Portland cement (31-34), but no study has investigated the antifungal effect of NEC. Our present data showed that the antimicrobial effect of NEC increased with incubation time. In addition, an increase in cement concentration resulted in an increase in the diameter of the inhibition zone.

The ineffectiveness of NEC, Portland cement, and MTA pastes against *E. faecalis* and a number of facultative and anaerobic bacteria reported in other studies indicates that if surgically treated root canals still contain this bacterium, these materials may not affect its growth and pathogenicity. In addition, the antibacterial effect of the test materials might only be temporary. Long-term studies are needed to investigate the effects of set materials against various bacteria commonly found in infected root canals.

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