

# Evaluation of the Antibacterial and Antifungal Effects of ProRoot MTA and Nano-fast Cement: An *In Vitro* Study

Fariborz Moazami<sup>1</sup>, Ahmad Gholami<sup>2</sup>, Vahid Mehrabi<sup>3</sup>, Yasamin Ghahramani<sup>4</sup>

## ABSTRACT

**Aim:** One of the most vital characteristics of an ideal root filling material is the capability to inhibit the growth of the microorganisms. Mineral trioxide aggregate (MTA) is one of the most used root repair materials, with approved antibacterial effect. A newly introduced root repair material is nano-fast cement (NFC) which should be investigated. The antibacterial and antifungal activities of NFC were evaluated in the present study.

**Materials and methods:** *Enterococcus faecalis* (PTCC 1394), *Escherichia coli* (ATCC 15224), and *Candida albicans* (PTCC 5027) were employed for the antimicrobial assessment. The following were the steps used to conduct the agar diffusion test (ADT): six agar plates were used. 0.5 McFarland concentration of each strain was cultured on two plates by a sterile cotton-tipped swab. Three holes with 5mm diameter were created on each plate. Freshly mixed cement was placed in the holes of the related plate. After two hours, the plates were incubated at 37°C for 24 hours. Then, the diameter of the growth inhibition zones were measured, and the mean values were used for the analysis. Direct contact test (DCT) was done by using the following steps: Freshly mixed materials were placed in the 96-well microtiter plate. 10 µL of each bacterial suspension was added to the tested cement. After one-hour incubation at 37°C, 245 µL of BHI broth was added to each well, and the plate was vortexed for 2 minutes. About 15 µL of this bacterial suspension was added to a new well which contained 215 µL of fresh medium. The kinetics of the bacterial outgrowth were measured by the microplate spectrophotometer hourly for 12 hours.

**Results:** No significant differences were observed between the diameters of the growth inhibition zones of MTA and NFC groups in ADT. In DCT, the MTA inhibits *E. coli* more effectively than NFC (*p* value < 0.001). Both cements had the same inhibitory effect on *E. faecalis* and *C. albicans*.

**Conclusion:** The MTA and NFC are almost equally effective against the tested microorganisms.

**Clinical significance:** The antibacterial characteristic of any dental material is an important matter. As well, the antibacterial efficacy of the NFC should be evaluated.

**Keywords:** Antibacterial, Antifungal, Mineral trioxide aggregate, Root repair material.

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## INTRODUCTION

Microorganisms play a significant role in the development of pulpal and periapical diseases and remain the main cause of endodontic treatment failure.<sup>1,2</sup> Success in endodontic treatment is influenced by proper debridement, thorough disinfection, and suitable obturation.<sup>3</sup> Most of the studies have shown that certain microorganisms such as *Enterococcus* spp., *Actinomyces* spp., *Propionibacterium* spp., yeasts, and *Streptococcus* spp. can be found in the previously root canal-filled teeth.<sup>4</sup> One of the most vital characteristics of an ideal root filling material is the capability to inhibit the growth of these microorganisms.

The mineral trioxide aggregate (MTA) is among the most utilized endodontic cements, which is used in pulp capping,<sup>5</sup> pulpotomy,<sup>6</sup> apexification,<sup>7</sup> root canal filling, and repairing root perforations.<sup>8</sup> Most of the studies that have evaluated the influence of MTA on microorganisms associated with endodontic diseases have reported conflicting results.<sup>9–11</sup> It is reported that gray and white MTA could inhibit the growth of *E. faecalis* and *S. sanguis*.<sup>9</sup> Also, some of the studies found that MTA is effective against *C. albicans*.<sup>12,13</sup> Torabinejad et al. in 1995 evaluated the effect of MTA on nine facultative bacteria and demonstrated that MTA did not inhibit *E. faecalis* and *E. coli*.<sup>5</sup> Although it is proven that MTA has positive characteristics such as biocompatibility,<sup>14,15</sup> providing an excellent seal,<sup>16,17</sup> minimal toxicity,<sup>18,19</sup> low solubility, and hard tissue formation,<sup>20</sup> it holds disadvantages such as long setting time,<sup>3</sup> inducing tooth discoloration,<sup>21</sup> difficult handling,<sup>22</sup> and high cost.

<sup>1,3,4</sup>Department of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>2</sup>Department of Pharmaceutical Biotechnology, School of Pharmacy, Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

**Corresponding Author:** Yasamin Ghahramani, Department of Endodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran, Phone: +98-711-6263193, e-mail: ghahramani.yas@gmail.com

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**Conflict of interest:** None

Recently, the researchers at Shiraz University of Medical Sciences manufactured a nano-calcium silicate-based cement named nano-fast cement (NFC), which has exhibited promising properties such as short setting time and simple handling.<sup>23</sup> Mineral trioxide aggregate and NFC consisted of almost similar chemical elements although the size of the particles is not the same. Further studies are compulsory to investigate the physical and biological aspects of this new biomaterial.

The purpose of this study was to evaluate and compare the antibacterial and antifungal activities of ProRoot MTA and NFC with ADT and DCT.

## MATERIALS AND METHODS

### Agar Diffusion Test

In this study, two experimental groups were utilized as follows: ProRoot MTA white-colored formula (Dentsply Tulsa Dental, Tulsa, Oklahoma, USA) and NFC (Sanat Avaran Vista, Shiraz, Iran). The antibacterial activity was evaluated employing two bacterial strains including *Enterococcus faecalis* (PTCC 1394) and *Escherichia coli* (ATCC 15224), and the antifungal activity was assessed using *Candida albicans* (PTCC 5027). These microorganisms were obtained from the Faculty of Pharmacy of Shiraz University of Medical Sciences. Six agar plates that contained Mueller Hinton Agar (HiMedia, India) medium were used. 0.5 McFarland concentration of each strain ( $1.5 \times 10^8$  bacteria) was prepared and cultured on the corresponding plates. Three wells (holes) were formed in each plate with sufficient distance from each other using a copper puncher with a diameter of 5 mm. The experimental materials were sterilized with ultraviolet germicidal irradiation and each mixed with a sterile spatula according to the manufacturer's instruction. The mixed materials were employed in the wells of each plate via a sterile amalgam carrier. All plates were preserved at room temperature for 2 hours to allow the prediffusion of the materials and then incubated (Jal Tajhiz Labtech. Co. Ltd., Tehran, Iran) at 37°C for 24 hours. The diameter of the growth inhibition zones was then determined using a ruler with 0.5-mm accuracy. The mean values of three wells were used for statistical analyzes. A comparison between the two cements was performed by Mann–Whitney U test, and SPSS ver. 16.0 software was used for the analysis.

### Direct Contact Test

Weiss et al.<sup>24</sup> have described this technique in detail. The DCT is based on the turbidimetric determination of bacterial growth in 96-well microtiter plates. The bottom and sides of each well were smeared to a height of 2 mm by freshly mixed test materials using a sterile amalgam carrier.

A 96-well microtiter plate has 12 columns. The first well of each column was covered with each tested material as the following sequence: MTA was placed in well numbers 1–3, NFC was applied in well numbers 7–9, and well numbers 4, 5, 6, 10, 11, 12 and were left empty. A 10  $\mu$ L of each microbial suspension was placed on the corresponding test material. After the incubation in a humid atmosphere at 37°C for one hour, the suspension liquid evaporated, ensuring direct contact between the whole bacteria and surfaces of the experimental materials. BHI broth (245  $\mu$ L) was added to each of the wells and the plate was gently vortexed for 2 min. About 15  $\mu$ L of the resultant bacterial suspension was then transferred to each well of B, C, and D rows that contained 215  $\mu$ L of fresh medium and again mixed for 2 minute. The kinetics of the bacterial outgrowth in each well of B, C, and D rows were then assessed hourly using a microplate spectrophotometer (PowerWave™ X52, BioTek Instruments Inc., Potton, UK) at 620 nm for 12 hours. Rows E, F, G, and H beneath the tested materials contained only microorganisms and BHI broth (control group). One-way ANOVA, *post hoc* Bonferroni test, and SPSS ver.160 software were used for statistical analysis.

## RESULTS

### Agar Diffusion Test

The growth of the microorganisms was inhibited in all experimental groups. Table 1 shows the means and standard deviations (mean  $\pm$  SD) of the growth inhibition zones which display the antibacterial

**Table 1:** Means and standard deviations (mean  $\pm$  SD) of the growth inhibition zones

Materials	Microorganisms		
	<i>E. faecalis</i>	<i>E. coli</i>	<i>Candida</i>
NFC	14 $\pm$ 1.7	10.3 $\pm$ 0.5	12 $\pm$ 1
MTA	11 $\pm$ 1	8.6 $\pm$ 0.5	11.6 $\pm$ 1.1
<i>p</i> value	0.1	0.1	0.7

and antifungal activities of experimental materials. After 24 hours of incubation, NFC demonstrated larger inhibition zones compared with MTA for all the tested microorganisms. Based on the results of the Mann–Whitney U test, there were no significant differences between the diameters of the growth inhibition zones in MTA groups compared with NFC ( $p \geq 0.05$ ).

### Direct Contact Test

The overall results of direct contact test (DCT) with different microorganisms are shown in Figure 1. There was a statistically significant difference between the control group and the tested cements, which indicates that both of the experimental materials inhibited the growth of three employed microorganisms.

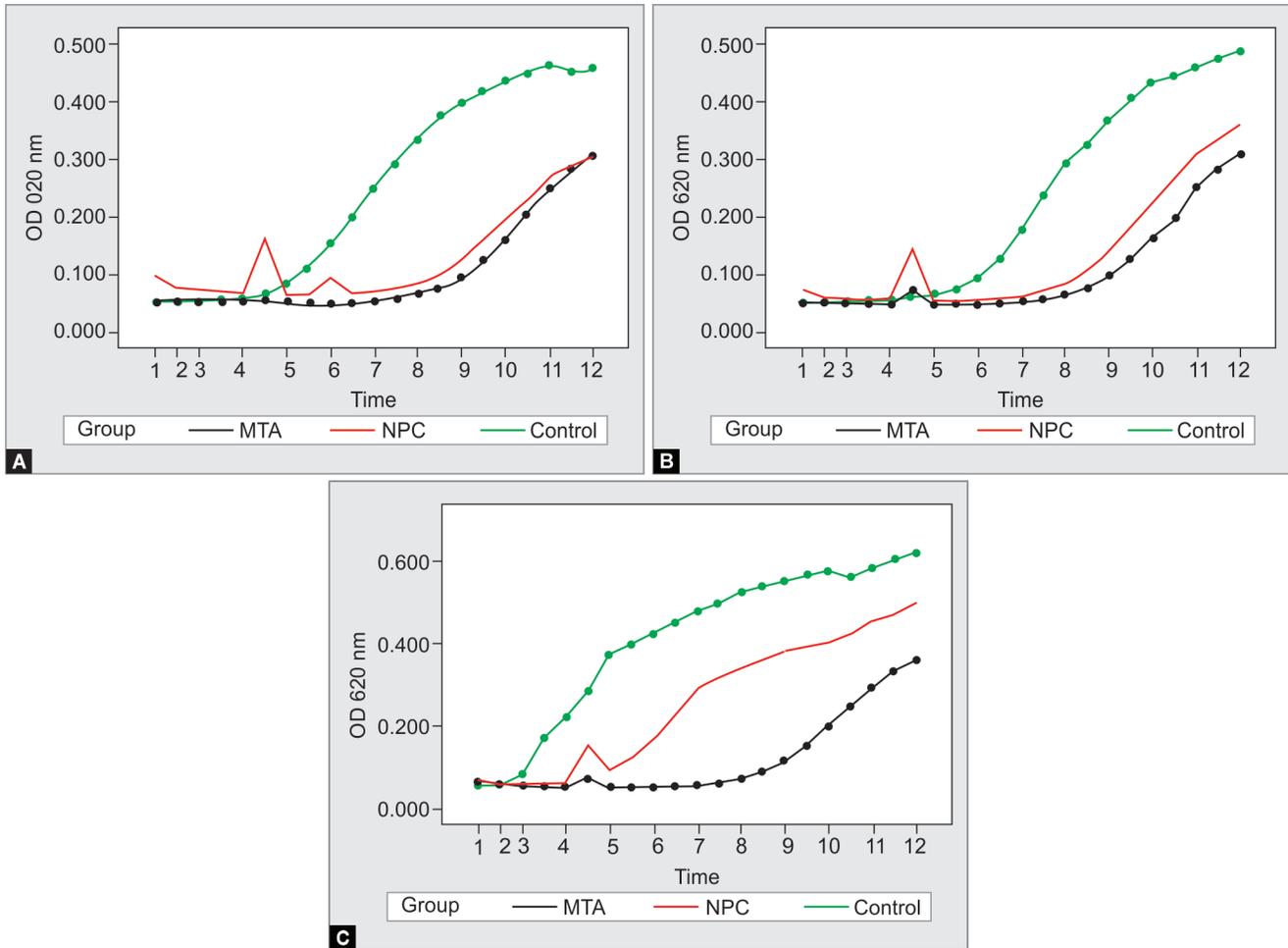
There was no statistically significant difference between the antibacterial and antifungal activities of MTA and NFC against *E. faecalis* and *C. albicans* in a 12-hour incubation period ( $p \geq 0.05$ ).

A significant difference was found between MTA and NFC against *E. coli*, which implies that MTA had a significantly greater inhibitory effect on the growth of *E. coli* than NFC ( $p < 0.001$ ).

## DISCUSSION

The microorganisms employed in this study were true endodontic pathogens<sup>25</sup> or were associated with cases that were resistant to therapy. *E. faecalis* is one of the most prevalent microorganisms found in the canals of the teeth with periapical periodontitis.<sup>26</sup> *E. faecalis* is resistant to some intra-canal medicaments and can survive in the root canal system of the obturated teeth. For those reasons, numerous studies assessing the antibacterial properties of endodontic cements had used these bacteria.<sup>25</sup> *C. albicans* is a part of the oral cavity's normal flora.<sup>27</sup> It has occasionally been found in primary root canal infections but seems to be more common in the root canals of obturated teeth in which the treatment has failed.<sup>28</sup> It has been demonstrated that *C. albicans* can invade dentinal tubules to variable degrees<sup>29,30</sup> and is highly resistant to some intra-canal medicaments like calcium hydroxide.<sup>31</sup> Another microorganism, which has been often found in an infected root canal, is *E. coli*,<sup>32</sup> a gram-negative facultative anaerobe. Due to the aforementioned reasons, a gram-positive (*E. faecalis*) and a gram-negative (*E. coli*) bacteria along with *C. albicans* were employed as the test microorganisms in this study.

The ADT is the most commonly applied method to assess the antimicrobial activity of endodontic cements;<sup>33,34</sup> however, the limitations of this method are well recognized. This technique is semiquantitative and does not distinguish between bactericidal and bacteriostatic effects of an agent. The results of ADT are vastly influenced by the solubility and diffusibility of the experimental agent through the agar. The DCT is a quantitative and reproducible method that simulates the contact of microorganisms with endodontic cements within the root canal system.<sup>24</sup> Both of these methods were utilized in the current study to achieve a better assessment of the antimicrobial effect of the experimental materials.



**Figs 1A to C:** (A) Optical density graphs of DCT on *E. faecalis*; (B) Optical density graphs of DCT on *C. albicans*; (C) Optical density graphs of DCT on *E. coli*. All the tests were measured over the 12-hour timeline

Previous studies have displayed conflicting results regarding the antimicrobial activity of MTA. Al Hezaimi et al. reported that MTA delayed or inhibited the growth of *E. faecalis*.<sup>9</sup> Bhavana et al. employed ADT and found that MTA is effective against *C. albicans*.<sup>12</sup> Sipert et al. reported that MTA is useful against *E. faecalis* but not against *E. coli*.<sup>11</sup> The result of the present study is in line with the results of Al Hezaimi et al. and Bhavana et al. and partially is in agreement with the results of Sipert et al. The partial agreement might be due to the MTA type (sealer) used in their study and the different sample sizes. Torabinejad et al. demonstrated that MTA did not inhibit *E. faecalis* and *E. coli*.<sup>5</sup> Also Estrela et al. stated that MTA did not show any antimicrobial activity against *E. faecalis* and *C. albicans*.<sup>35</sup> The differences between the methodologies can lead to different results. The current study used DCT too, though Torabinejad et al. and Estrela et al. used ADT. Furthermore, Estrela et al. used different agar mediums.

In the current study, NFC revealed similar antimicrobial activity as compared to MTA. ProRoot MTA and MTA-like materials release calcium hydroxide after contacting the tissue fluids or water. The decomposition of calcium hydroxide into calcium and hydroxide ions creates an alkaline pH which is responsible for the antimicrobial activity of the related cements.<sup>36</sup> NFC, which is an MTA-like material, was manufactured at Shiraz University. The particle sizes of the powder were intentionally decreased. NFC shows a short setting

time, and in one study, the addition of distilled water and multi-walled carbon nanotube to the chemical structure of NFC triggered an improvement in the physical properties of the cement.<sup>23</sup>

In the ADT, the diameter of the growth inhibition zones of NFC was relatively larger than MTA which could be due to the nano-sized particles of NFC producing higher surface-to-volume ratio and faster rate of diffusion through the agar. There was no statistically significant difference between the diameters of the growth inhibition zones of the tested material after 24 hours.

The results of the DCT showed no statistically significant difference between the tested cements against *E. faecalis* and *C. albicans*, which is similar to the results of the ADT. However, the DCT revealed that MTA inhibited *E. coli* more effectively than NFC in 12 hours. Possibly the NFC needs more time (*i.e.*, 24 hours) to show the maximum inhibitory effect on *E. coli*. As mentioned before, the ADT was conducted in 24 hours, but in the DCT, densitometric readings were taken within 12 hours. Previous studies had performed the DCT in a maximum of 17-hour period.<sup>24,37</sup>

## LIMITATIONS OF THE STUDY

The cost of ProRoot MTA and its accessibility were the main limitations of the current study. Besides, the *in vitro* method of the study is a limitation itself. Different kinds of material diffusibilities

in the agar test and colorimetric nature of the DCT are also the study limitations.

## CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that MTA and NFC demonstrate almost similar antimicrobial properties against *E. faecalis*, *E. coli*, and *C. albicans*. These results could be temporary and further studies are required to investigate the precise antimicrobial effect of the NFC on endodontic pathogens.

## CLINICAL SIGNIFICANCE

The antibacterial characteristic of any dental material is an important matter. As well, the antibacterial efficacy of the new NFC should be evaluated to be used in the future investigations in animal and human models.

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