CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1087424

Available online at: http://www.iajps.com Research Article

ANTIBACTERIAL ACTIVITY OF MINERAL TRIOXIDE AGGREGATE, NEW ENDODONTIC CEMENT, RETRO MTA AND ORTHO MTA AGAINST COMMON ENDODONTIC PATHOGENS

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Abstract:

Introduction: *Mineral trioxide aggregate [MTA] has extensive applications in endodontic treatment. Retro MTA, Ortho MTA and new endodontic cement [NEC] were also introduced to overcome the limitations of MTA. This study sought to compare the antibacterial activity of MTA, NEC, Ortho MTA and Retro MTA against common endodontic pathogens.*

Materials and Methods: *This in vitro, experimental study was conducted on standard strains of Streptococcus mutans, Enterococcus faecalis, Actinomyces viscosus and Escherichia coli. Antibacterial activity of the materials was tested using the disc diffusion method. Wells were created in Mueller Hinton agar plates inoculated with bacterial suspensions with 0.5McFarland concentration. The plates were incubated at 35°C for 18 hours and diameter of the growth inhibition zones was measured by a ruler. The data were analyzed using the Kruskal Wallis test. Pairwise comparisons were done using Bonferroni test.*

Results: *The mean diameter of the growth inhibition zone of E. coli was 2.916, 10.833, 10.333 and 7.25mmin presence of MTA, NEC, Retro MTA and Ortho MTA, respectively [P<0.05]; these values were 5.083, 8.916, 7.916 and 7.416mm, respectively for E. faecalis [P=0.17], 6.0, 8.916, 7.333 and 8.333mm, respectively for S. mutans [P<0.24] and 1.916, 8.083, 0 and 0.666, respectively for A. viscosus [P<0.05]. Antibacterial activity of all four materials was the same against E. faecalis and S. mutans. All materials showed limited antibacterial activity against A. viscosus except for NEC.*

Conclusion: *Retro MTA and Ortho MTA had acceptable antimicrobial activity against common endodontic pathogens [except for A. viscous] and thus, they can be used as an alterative to MTA in the clinical setting given that their other properties are confirmed to be optimal.*

Key words: *Anti-Bacterial Agents; Mineral Trioxide Aggregate; Root Canal Therapy*

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Please cite this article in press as Mohammad Esmaeilzadeh et al., ***Antibacterial Activity of Mineral Trioxide Aggregate, New Endodontic Cement, Retro MTA and Ortho MTA against Common Endodontic Pathogens****, Indo Am. J. P. Sci, 2017; 4(12).*

INTRODUCTION:

Microorganisms play a role in failure of endodontic treatments [1, 2]. Thus, it is imperative to eliminate microorganisms from the root canal system. However, some bacteria may remain in the root canal system [3]. Thus, success of root canal treatment depends on successful elimination of bacteria and seal of the root canal system [4]. Since none of the available root canal filling materials can provide a hermetic seal, they must have antibacterial properties to prevent infection. Several root canal filling materials have been introduced with such properties but none of them are ideal [5].

ProRoot MTA is among the most commonly used dental materials for perforation repair and endodontic treatments [6]. It is available in two forms of white and gray MTA and is composed of 75% Portland cement, 20% bismuth oxide and 5% gypsum. MTA was first introduced as a root end filling material but it was later used for pulp capping and pulpotomy treatments, for the induction of apical barrier formation in open apex teeth, perforation repair and root canal filling [7, 8]. MTA has favorable properties such as providing an excellent seal, biocompatibility, low toxicity, low solubility, hard tissue formation, high pH and radiopacity [9-11]. However, it also has drawbacks such as long setting time [about four hours], tooth discoloration, difficult handling and high cost [12]. Evidence of antibacterial properties of MTA also exists; however, these properties have not been well confirmed [6].

To overcome the shortcomings of MTA, Ortho MTA and Retro MTA were later introduced in 2010. Due to their unique properties, these products gained high popularity among dental clinicians. Elimination of Portland cement and its replacement with a new generation of nano materials resulted in elimination of toxic compounds and heavy metals from the composition of MTA. Also, iron content, which was mainly responsible for tooth discoloration, was minimized. These modifications significantly decreased the setting time of MTA as Retro MTA sets in 180 seconds and Ortho MTA sets in 150 minutes. These products are supplied in the form of powder comprising of hydrophilic particles that set in presence of water and form an impermeable barrier with a primary pH of as high as 12.5. These materials are biologically bioactive and induce regeneration of the injured periodontium [13]. However, the antimicrobial properties of these products have not yet been evaluated [13].

New endodontic cement is a recently introduced material composed of several calcium compounds. It recently gained ISO approval for its physical properties [1]. Clinical applications of NEC, also known as calcium enriched mixture [CEM] cement, are similar to those of MTA and it even showed superior results to MTA for pulp capping treatment of teeth [12]. It has insignificant cytotoxicity for different cell lines and has shown less dye penetration [leakage] than MTA [14]. Working time, pH and dimensional changes of MTA and NEC are the same while NEC has better handling and can produce hydroxyapatite by releasing calcium and phosphate ions [15]. The results of previous studies on the antimicrobial properties of NEC have been controversial [1, 13].

Agar diffusion test is a standard method for assessment of antibacterial properties of materials in vitro. It is extensively used for accurate and direct comparison of antibacterial effects of materials [3, 6].

Microorganisms such as E. faecalis, E. coli and A. viscosus are dominant in treatment-resistant periapical lesions [1]. Enterococcus faecalis and A. viscosus are strong microorganisms that contaminate the root canal system and play a role in primary endodontic treatment failures [15-18]. Escherichia coli has also been isolated from the root canal system [19] and is a common microorganism in odontogenic infections [20]. Streptococcus mutans is also responsible for primary periapical lesions and secondary infections [21].
Considering all the above, this study sought to compare the antibacterial activity of MTA, NEC, Retro MTA and Ortho MTA against pathogenic microorganisms commonly involved in endodontic infections.

MATERIALS AND METHODS:

In this in vitro experimental study, standard strains of S. mutans, E. coli, E. faecalis and A. viscosus were used for assessment of antibacterial activity of MTA, NEC, Retro MTA and Ortho MTA using agar diffusion test. Sample size was calculated to be 48 samples in each group [a total of 192] according to a study by Zarrabi et al, in 2009 [1] and considering 95% confidence interval and 80% power of the study using R software. Four experimental groups namely the MTA [Angelus, Londrina, PR, Brazil], NEC [calcium enriched mixture], Retro MTA [BioMTA, Seoul, Korea] and Ortho MTA[BioMTA, Seoul, Korea] were designed. Standard strains of S. mutans, E. faecalis, E. coli and A. viscosus were obtained from the Microbiology Department of Hamadan University of Medical Sciences. Mueller Hinton agar medium was obtained and wells were created in the gel using a copper puncher with a diameter corresponding to that of discs [6mm]. Five wells were created in each plate with adequate distance from each other. Next, 0.2g of each material was weighed and diluted with one to five drops of solvent. Next, 0.5McFarland standard concentration of each strain [1.5×10^8 bacteria] was prepared and cultured on Mueller Hinton agar. Different concentrations of each material were poured into wells and the plates were incubated at

35°C for 18 hours. The diameter of the growth inhibition zones was then measured using a ruler with 0.5mm accuracy [Figure 1]. This test was repeated three times and the mean values were used for statistical analyses.

The data were analyzed using SPSS version 21. The mean and standard deviation of growth inhibition zones of each bacterial strain in presence of different materials were measured and reported. Kolmogorov-Smirnov test was used to assess the distribution of the growth inhibition zone data, which showed that the data did not have a normal distribution [$P<0.05$]. Thus, the Kruskal-Wallis test was used to compare the growth inhibition zones among different bacteria and materials. In case of presence of significant differences found with the Kruskal-Wallis test, the Bonferroni test was used for pairwise comparisons. $P\leq0.05$ was considered statistically significant.

RESULTS:

The results showed that the mean diameter of the growth inhibition zone of E. coli was 2.916, 10.833, 10.333 and 7.25mm in presence of MTA, NEC, Retro MTA and Ortho MTA, respectively. The smallest and the largest growth inhibition zones were caused by MTA and NEC, respectively. According to the non-parametric Kruskal Wallis test, significant differences were noted in the mean growth inhibition zone of E. coli among the four materials [P=0.00, Diagram 1].

The mean diameter of the growth inhibition zone of E. faecalis was 5.083, 8.916, 7.916 and 7.416 mm in presence of MTA, NEC, Retro MTA and Ortho MTA, respectively. The smallest and the largest growth inhibition zones were caused by MTA and NEC, respectively. According to the non-parametric Kruskal Wallis test, the difference in this regard among the four materials was not significant [P=0.17, Diagram 2].

The mean diameter of the growth inhibition zone of S. mutans was 6.0, 8.916, 7.333 and 8.333mm in presence of MTA, NEC, Retro MTA and Ortho MTA, respectively. The smallest and the largest growth inhibition zones were caused by MTA and NEC, respectively. According to the non-parametric Kruskal Wallis test, the difference in this regard among the four materials was not significant [P=0.24, Diagram 3].

The mean diameter of the growth inhibition zone of A. viscosus was 1.916, 8.083, 0 and 0.666 in presence of MTA, NEC, Retro MTA and Ortho MTA, respectively. The smallest and the largest growth inhibition zones were caused by Retro MTA and NEC, respectively. Retro MTA had no antibacterial activity against A. viscosus. According to the non-parametric Kruskal Wallis test, significant differences were noted in the mean growth inhibition zone of A. viscosus among the four materials [P=0.000, Diagram 4].

Based on the results of the Mann Whitney test with Bonferroni adjustment [level of significance was adjusted to $\alpha=0.00625$], a significant difference was found in the diameter of the growth inhibition zone of E. coli between Retro MTA and MTA [$P<0.0001$] but no other significant differences were noted in this regard between other groups. Moreover, the difference between NEC and Retro MTA [$P<0.001$] and NEC and Ortho MTA [$P<0.005$] in terms of growth inhibition zone of A. viscosus was significant. The results of pairwise comparisons of the groups are shown in Table 1.

Table 1: Pairwise comparisons of the materials for bacteria with significant differences in the mean diameter of growth inhibition zone

Pairwise comparisons	E. coli P value	A. viscosus P value	
NEC-Retro MTA	0.219	0.001*	**α=0.006 [Bonferroni adjustment]**
NEC-MTA	0.001*	0.014	
NEC-Ortho MTA	0.039	0.005*	
Retro MTA-MTA	0.000*	0.319	
Retro MTA-Ortho MTA	0.068	0.755	
MTA-Ortho MTA	0.028	0.514	

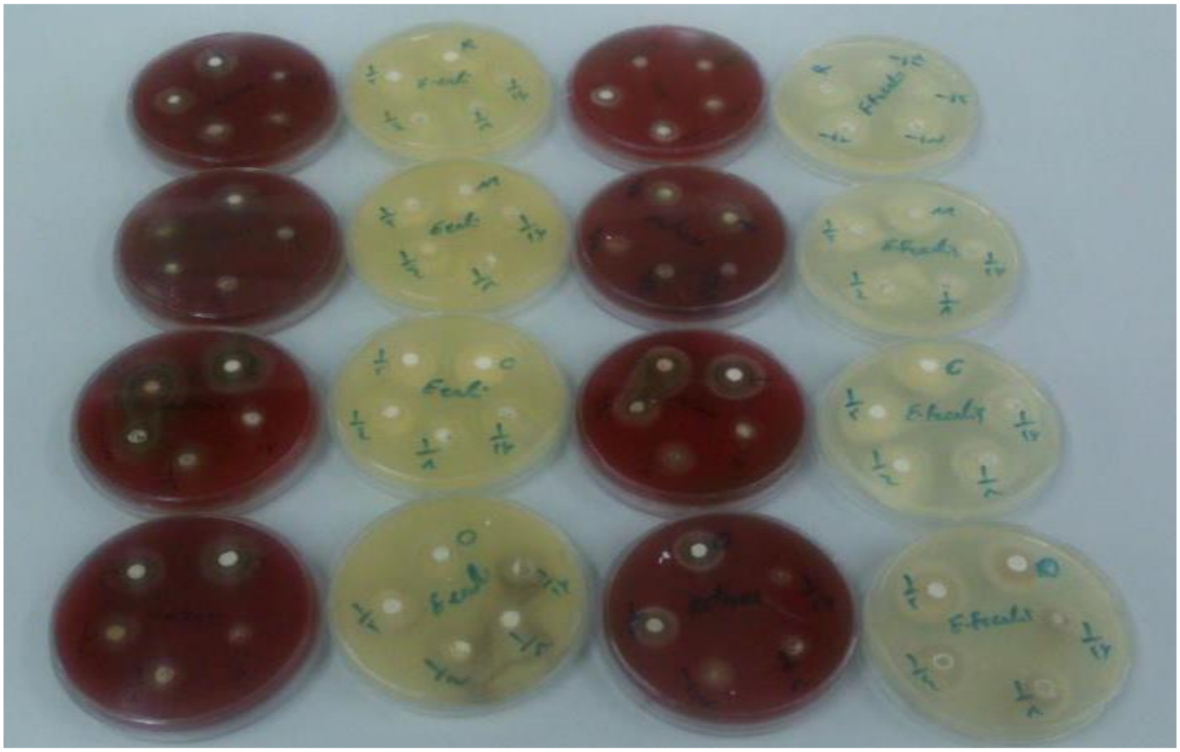

Figure 1: Growth inhibition zones of the four bacterial strains in presence of different concentrations of the materials

Diagram 1. The mean diameter of E. coli growth inhibition zone in the four groups [in millimeters]

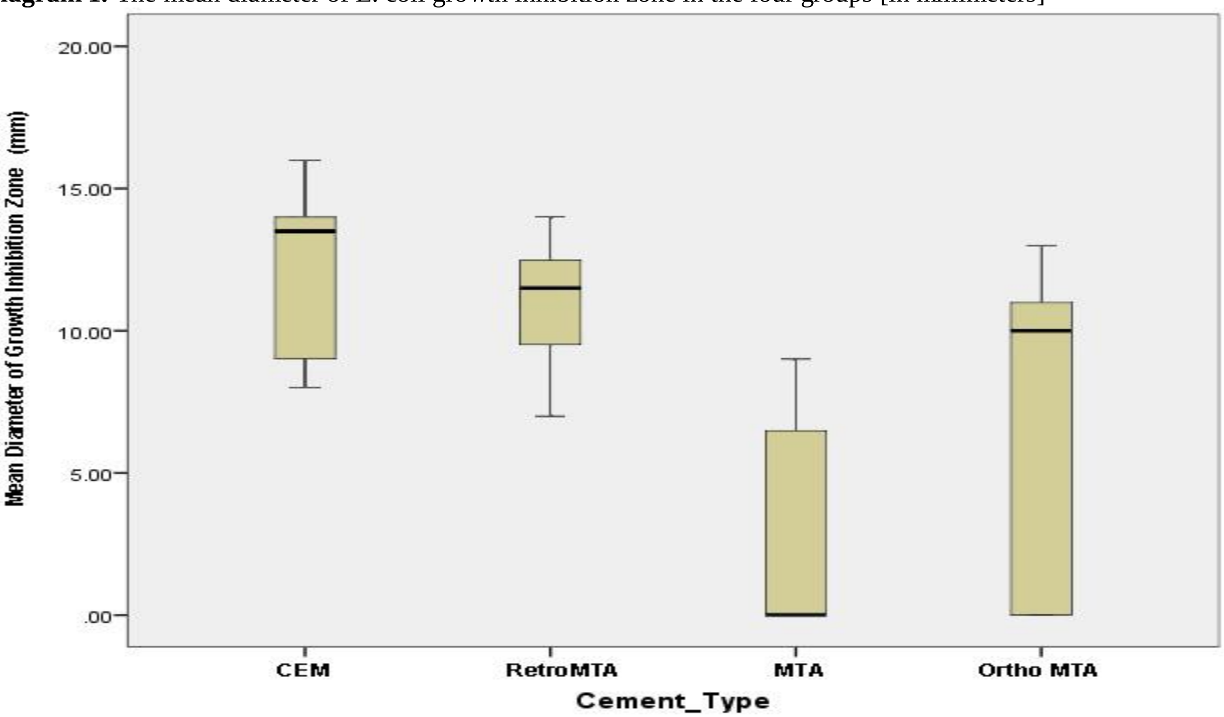

Diagram 1. The mean diameter of E. coli growth inhibition zone in the four groups [in millimeters]

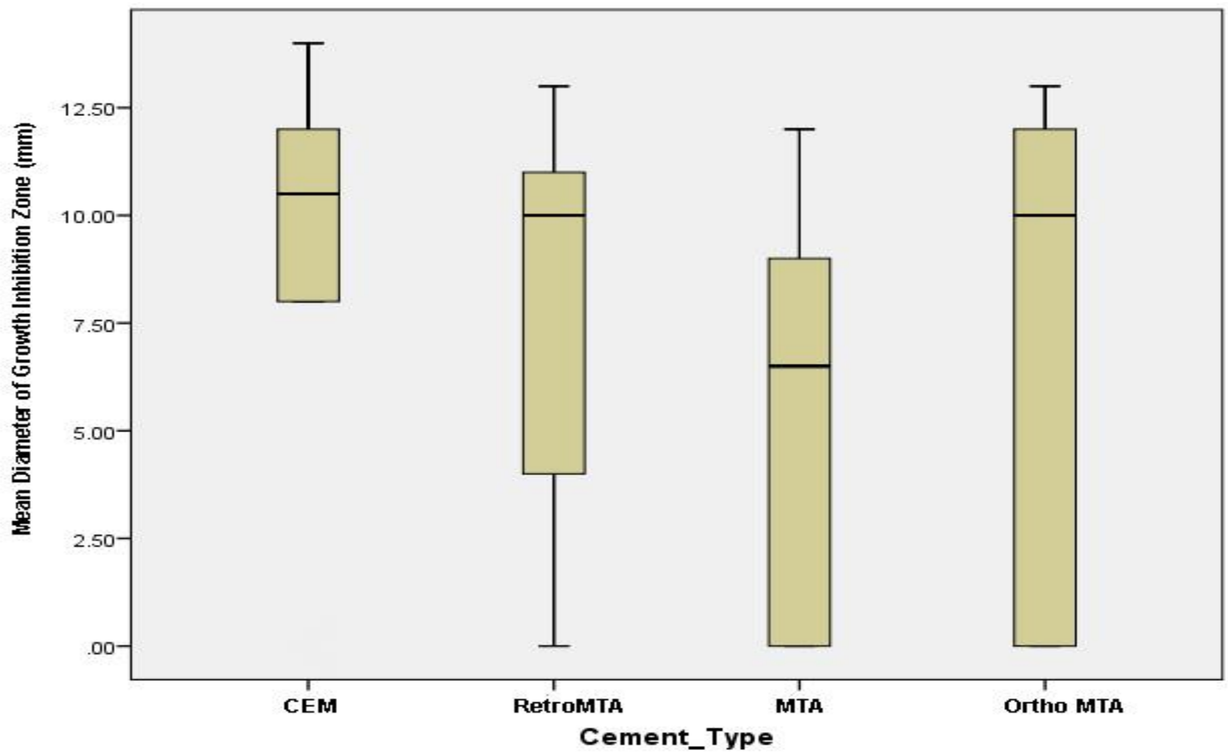

Diagram 2. The mean diameter of E. faecalis growth inhibition zone in the four groups [in millimeters]

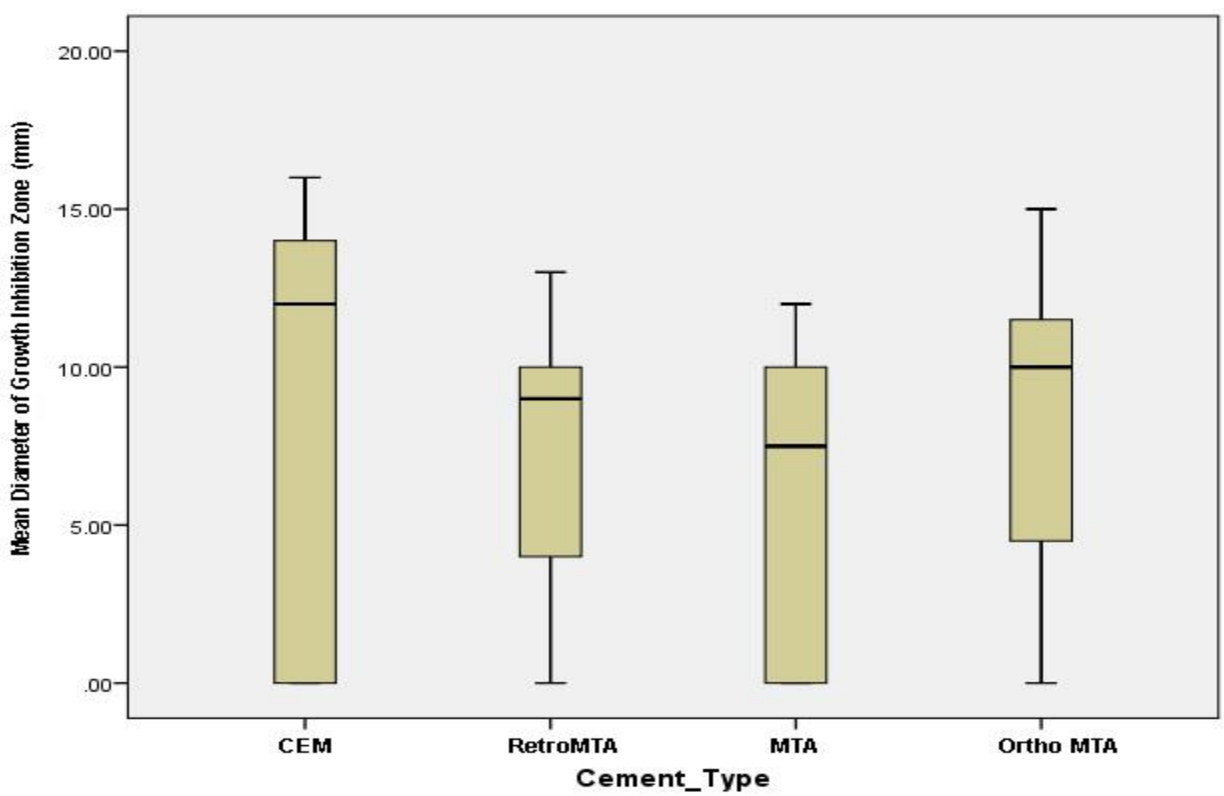

Diagram 3. The mean diameter of S. mutans growth inhibition zone in the four groups [in millimeters]

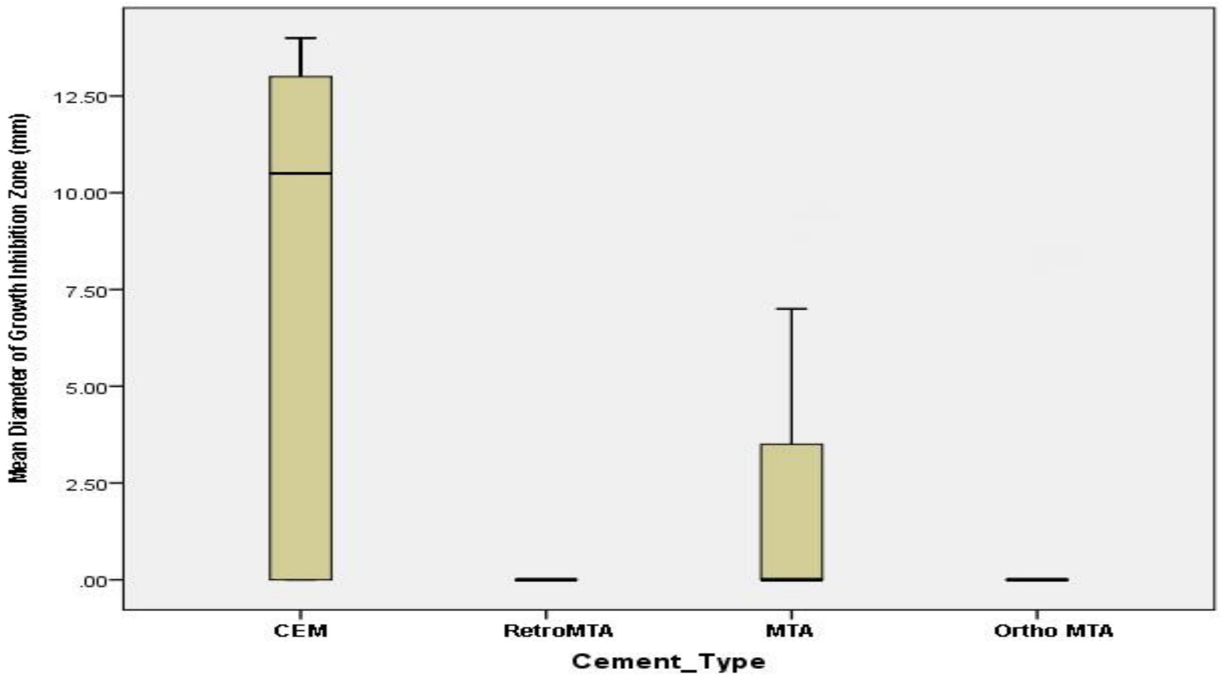

Diagram 4. The mean diameter of E. viscosus growth inhibition zone in the four groups [in millimeters]

DISCUSSION:

Based on the results, it appears that NEC had stronger antibacterial properties than other understudy materials. Alkaline metal oxides, calcium oxide, calcium hydroxide, calcium phosphate and calcium silicate are the main constituents of NEC. When NEC is transferred to agar plates, $Ca[OH]_2$ is released from the calcium and hydroxyl ions and increases the pH and concentration of calcium. This mechanism may somehow increase the antimicrobial properties of NEC in vitro. On the other hand, antimicrobial compounds in the composition of NEC have better diffusion properties compared to those in other materials such as MTA. This process also plays a role in enhanced antibacterial properties of NEC [3]. Asgary and Akbari Kamrani in a similar study in 2008 showed that the largest diameter of the growth inhibition zone was formed around NEC [22]. Thus, they concluded that NEC had antimicrobial activity to the level of calcium hydroxide; which was in agreement with our findings.

Zarrabi et al, in 2009 showed that antimicrobial activity of NEC was superior to that of MTA and Portland cement against E. faecalis, E. coli, S. mutans, C. albicans and A. viscosus [1]. Their methodology was the same as ours, which explains the similarity between the results of the two studies. Razmi et al, in 2013 evaluated the antibacterial activity of NEC and ProRoot MTA against E. faecalis and showed that NEC and MTA had optimal antibacterial activity against E. faecalis, which was in agreement with our results [23].

In total, it appears that the antimicrobial efficacy of NEC is higher than that of MTA in vitro. However, further studies on the antibacterial efficacy of NEC in vivo are still required. Also, the composition of NEC and the effects of its components in vivo can be interesting topics for future research in this field.

In the current study, MTA had minimal antibacterial activity against E. coli, E. faecalis and S. mutans; however, the difference of MTA with other materials in this regard was not significant for E. faecalis and S. mutans. In other words, MTA had antibacterial activity similar to that of NEC, Retro MTA and Ortho MTA against E. faecalis and S. mutans.

At present, MTA is the material of choice for root perforations, pulp capping and retrograde root canal filling [7, 8, 24]. Previous studies have reported controversial results regarding antibacterial properties of MTA. For instance, antimicrobial activity of MTA has been reported to be insignificant in some [25, 26] and sufficiently high against E. faecalis in some other studies [4, 27]. However, it should be noted that antibacterial

properties of MTA depend on its concentration and the preparation method [28].
Using agar diffusion test, Sipert et al, in 2005 demonstrated that MTA inhibited the growth and proliferation of C. albicans, S. aureus, E. faecalis, S. epidermidis and S. aeruginosa in vitro [29]. In another study, Ribeiro et al, in 2010 reported adequate antimicrobial activity of MTA against E. coli in aerobic conditions following induction of reactive oxygen species [30]. According to Riberio et al, in 2006 E. faecalis was susceptible to gray MTA [31]; they explained that oxygen-rich environment boosted the antimicrobial activity of MTA.

In a study by Luczaj-Cepowicz et al, in 2008, white ProRoot MTA caused larger growth inhibition zones of S. salivarius and S. sanguinis compared to white MTA Angelus and both materials showed antibacterial properties against S. mutans, S. sanguinis and S. salivarius [32]; these results were in accordance with our findings.

Antimicrobial activity of MTA is correlated to its high pH and release of materials that can be well diffused in the medium [33]. Torabinejad et al, in 1995 reported that the primary pH of MTA was 10.2, which increased to 12.5 after three hours [34]. A rise in the pH to 12 further inhibited the growth of microorganisms [35].

Based on the results of the current study, Ortho and Retro MTA had acceptable antibacterial activity against the bacterial strains [except for A. viscosus], which was slightly lower than that of NEC. The diameter of the growth inhibition zones of E. coli, S. mutans and E. faecalis caused by these materials was almost the same and higher than that of MTA. However, Ortho and Retro MTA had very limited activity against A. viscosus and Retro MTA had no antibacterial activity against A. viscosus.

The properties of MTA improved in Ortho and Retro MTA. These types of bioceramic MTA are devoid of heavy metals, have high fracture strength and the shortest setting time, cause no discoloration and have high radiopacity and thus, are suitable for endodontic and periodontal treatments. These products are composed of hydrophilic particles that set in presence of water and form an impermeable barrier. Their primary pH is as high as 12.5 [13]. This increase in pH explains their bactericidal properties. Ortho MTA was first introduced as a retrograde root canal filling material. It has suitable dentinal tubule sealing properties and has insignificant expansion [0.09%][36]. The manufacturer claims that Retro MTA does not contain heavy metals such as Cr, As, Ni, Fe, Bi or Cd and has insignificant toxicity [37]. Its short setting time [about 150 seconds] is suitable for single visit treatments. To the best of authors' knowledge, no previous study has evaluated the antimicrobial properties of Retro and Ortho MTA.

Based on the results of the current study, the diameter of the growth inhibition zone was different for different microorganisms. The largest growth inhibition zone belonged to E. coli in presence of NEC and Retro MTA and S. mutans in presence of MTA and Ortho MTA. In the study by Zarrabi et al, in 2009, the largest growth inhibition zone was seen in A. viscosus, which was different from our results [1]. Moreover, Al-Hezaimi et al, in 2006 reported that MTA had antimicrobial activity against E. faecalis and S. sanguinis, which was in accordance with our results [4]. Based on the current results, MTA had the highest antimicrobial activity against S. mutans and had less antimicrobial efficacy against E. coli, E. faecalis and A. viscosus. A previous study indicated that MTA had antimicrobial activity against E. coli [3] while some other studies reported no antimicrobial activity against E. coli [26, 34]. The controversy in this regard among the results of different studies may be due to the amount of available nutrients, oxidative stress, incubation time, assessment methods and different methodologies.

In the current study, agar diffusion test was used for assessment of antimicrobial efficacy of materials, which is the most commonly used technique for assessment of antimicrobial activity of materials in vitro [35]. Difference in the agar plate, diffusion ability of growth inhibiting substances, bacterial strains and their cell density may all affect the formation of growth inhibition zones [25]; thus, these factors must be well controlled for in order to increase the reliability and reproducibility of the results. On the other hand, agar diffusion test cannot differentiate between the bactericidal and bacteriostatic properties of materials. Moreover, creation of a growth inhibition zone depends not only on the effect of tested materials, but also on their pattern of diffusion in the medium [38].

Microorganisms tested in the current study are common culprits responsible for endodontic and periapical lesions; E. faecalis and A. viscosus infect the root canal system [16, 17] and play a role in endodontic failures and primary endodontic infections [18]. Also, E. coli has been isolated from the root canals and is a standard microorganism used for antimicrobial testing [19].

All four materials tested in the current study showed almost similar antimicrobial activity against E. faecalis and S. mutans with no significant difference; S. mutans is the main microorganism responsible for tooth decay and E. faecalis has been dominantly isolated from endodontic infections [39]. Due to high resistance to

antimicrobial agents, the latter is commonly used in such studies [39]. The odds of presence of E. faecalis in endodontically failed teeth are nine times higher than those of other strains. When the pH of calcium hydroxide medicament drops, resistance of E. faecalis to the medicament significantly increases [35]. Moreover, E. faecalis strains can stay alive for long periods of time inside dentinal tubules. Due to the importance of these microbial strains, they were used in the current study along with less common microorganisms such as E. coli.
According to Zarrabi et al, in 2009 [1] and since the objective of the current study was to assess the bactericidal properties of these materials, there was no need for an antibiotic control group. On the other hand, comparison of materials with antibiotics is often done when the materials to be tested have strong antibacterial properties and diffusion ability, which obviously was not the case in our study.

However, it should be noted that this study had an in vitro design and these materials may show different antimicrobial properties in the clinical setting. In the oral environment, saliva changes the pH and dilutes the materials. Also, oral temperature is variable and differs from that of incubator. Presence of blood and variable oxidation and reduction potential in the oral cavity also affect the results [40]. Last but not list, in the laboratory setting, antimicrobial agents are constantly in contact with the microorganisms in test tubes or plates while in the oral cavity, these materials are washed out of the mouth within a few seconds or are neutralized by the saliva components.

Future studies are required to confirm the accuracy of these findings by other methods such as the direct contact technique. Also, assessment of the substantivity of these materials and their antibacterial properties in the clinical setting can be an interesting topic for future research.

CONCLUSION:

Retro MTA and Ortho MTA had acceptable antimicrobial activity against common endodontic pathogens and can be used as an alterative to MTA in the clinical setting given that their other properties are confirmed to be optimal.

Conflict of interest: None

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