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Evaluation and comparison of four types of bio-ceramic materials AGM MTA, Ortho MTA, pro root MTA and Cem cement in oral and dental health

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Abstract

Background and objectives Mineral Trioxide Aggregate (MTA) is one of the main retrograde filling materials that is used today as a root end filling material and perforation repair material. This study was conducted with the aim of investigating the antibacterial and antifungal properties of four types of bio-ceramic materials, AGM MTA, Ortho MTA, Pro root MTA and Cem cement for oral and dental health.

Methods In this study, the antibacterial activity of four types of bio-ceramic materials against two bacterial strains of *Enterococcus faecalis* (ATTC 29212), *Escherichia coli* (ATTC 35318) and antifungal activity against *Candida albicans* (ATTC 10231) were investigated using the well diffusion method.

Results In the context of the relationship between the type of microorganism and the diameter of the growth inhibitory zone for each type of bio-ceramic material, there was no significant difference for *Enterococcus faecalis*, and a significant difference was observed for *Escherichia coli* and *Candida albicans* ($p < 0.05$).

Conclusion The results show that each of the bio-ceramic materials AGM, Pro root, Cem cement and Ortho have antibacterial and antifungal properties. AGM MTA bio-ceramic material on *Candida albicans* fungus and Ortho MTA bio-ceramic material had the most effect on Es*cherichia coli* bacteria. Therefore, the mentioned bio-ceramic materials can play a significant role in oral and dental health by providing a suitable material for restoration.

Keywords Oral health, Ceramics, *Enterococcus*, *Escherichia coli*, *Candida albicans*

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Introduction

Perforation is defined as an unintentional connection between the root canal and the external surface of the root with the periodontal ligament, which can be caused iatrogenically during endodontic treatments or due to deep caries, ultimately influencing the long-term prognosis for the tooth [1]. Immediate diagnosis and treatment of perforations leads to a better prognosis for them. The success of repairing perforations depends on proper filling and regeneration of periodontal fibers [2]. In addition, treatment is influenced by the size, location, and timing of the perforation, as well as the ability of the material to close these defects [3]. The choice of the type of restorative material is another important factor in the prognosis of perforation treatment, and the degree of tissue compatibility and the filling power of the restorative material are two important influencing factors [4]. The best material for sealing perforations is a material that is non-toxic, non-absorbable, radiopaque, bactericidal, and can create a strong flood [5]. Different materials are used to close perforations, Mineral Trioxide Aggregate (MTA) has good sealing power and tissue compatibility [6]. MTA, a material developed in 1993 at Loma Linda University, is an alkaline substance used for root-filling purposes [7]. This substance has low cytotoxicity and antibacterial effects, it is also compatible with tissue and causes osteogenesis and odontogenesis [8]. MTA is composed of 65% by weight of calcium oxide and 21% by weight of silica (silicon dioxide), as well as amounts of alumina (Al_2O_3), iron oxide (Fe_2O_3), and gypsum ($CaSo_4.2H_2O$). Bismuth oxide (Bi_2O_3) has also been used in this material to create radioactivity. MTA powder is hydrated by adding water and forms a colloidal gel that hardens in about four hours [9]. MTA contains calcium oxide when mixed with water, forms calcium hydroxide and increases the pH by dissociating calcium and hydroxide ions. The antimicrobial effect of MTA is attributed to its high initial pH of 10.2, which reaches 12.5 within 3 h [3]. One of its key characteristics is the ability to harden in the presence of moisture, creating suitable physical properties to serve as a barrier against pathogenic microorganisms [10]. Calcium-enriched mixed cement (Cem cement) contains calcium compounds. This material has good biocompatibility and can induce hard tissue, including hydroxyapatite [11]. The prominent feature of this material is its easier clinical application, antibacterial and antifungal effect, and filling ability comparable to MTA [12]. If a pathological lesion cannot be fixed by root canal treatment or if re-treatment fails, root canal surgery methods are used. An ideal retrograde filler material should be biocompatible with the tissues it contacts, have adequate flooding, allow or stimulate bone growth, and exhibit antimicrobial activity. Retrograde filling materials cannot create a complete flood. Accordingly, microscopic spaces remain between the retrograde cavity and the filling material through which microorganisms and their products may penetrate the root canal. As a result, the antimicrobial activities of the materials used as retrograde fillers are essential to be beneficial. MTA is one of the main retrograde filling materials that is often used today [13]. *Enterococcus faecalis* is one of the most common microorganisms found in tooth canals with periapical periodontitis, which is resistant to some intracanal drugs and can survive in the root canal system of treated teeth. *Candida albicans* is part of the natural flora of the oral cavity. This fungus is sometimes seen in early root canal infections, but it seems more common in root canals of avulsed teeth where treatment has failed. *Albicans* can attack dentin tubules and have high resistance to some intracanal drugs such as calcium hydroxide. *Escherichia coli* is a Gram-negative facultative anaerobic bacterium that is often found in infected root canals [14]. These microorganisms are found with greater frequency in cases of prolonged treatment, flare-ups, and failed treatments and can enter the root canal system before or after treatment and cause secondary infection [15].

Therefore, the purpose of our study was to investigate the antibacterial and antifungal properties of four types of bio-ceramic materials: AGM MTA, Ortho MTA, Pro root MTA, and Cem Cement, so that we can reduce the amount of damage caused to patients by providing a suitable material for restoration. The because AGM MTA is a new Iranian material, if this material is effective compared to other mentioned materials, many problems of dentists can be solved, including the unavailability of other bio-ceramics, and national production can be supported and achieve valuable results.

Materials and methods

The present research was a laboratory study that was conducted at the level of Qom province in the Faculty of Dentistry of Qom University of Medical Sciences in 2022. The statistical population under study was bio-ceramics of Mineral Trioxide Aggregate (MTA) with the appropriate consistency of the mentioned brands.

In this laboratory study, the antibacterial activity of bioceramic materials against two bacterial strains of *Enterococcus faecalis* (ATTC 29212) and *Escherichia coli* (ATTC 35318) and their antifungal activity against *Candida albicans* (ATTC 10231) were investigated. Microorganisms were obtained from the Bacteriology and Mycology Laboratory of Qom University of Medical Sciences. A concentration of 0.5 McFarland was prepared from each strain and cultured on Mueller Hinton agar culture (MHA) medium. In each of these plates, four wells with a diameter of 5 mm were created with a sufficient distance from each other according to a standard method previously reported by Jan Hudzicki [16].

Table 1 The diameter of the growth inhibiting zone of microorganisms after 24 h

bacteria and material names		Minimum	Maximum	Average	*p*-value
Enterococcus faecalis	AGM	8 mm	8.5 mm	8.3750 mm	0.410
	Pro root	7 mm	9 mm	7.8750 mm	
	Ortho	8 mm	9 mm	8.3750 mm	
	Cem cement	8 mm	8 mm	8.0000 mm	
	Total	7 mm	9 mm	8.1563 mm	
Escherichia coli	AGM	9 mm	9 mm	9.0000 mm	0.049
	Pro root	7.5 mm	12 mm	10.6250 mm	
	Ortho	10 mm	12.5 mm	10.8750 mm	
	Cem cement	80 mm	9 mm	8.5000 mm	
	Total	7.5 mm	12.5 mm	9.7500 mm	
Candida albicans	AGM	8 mm	9.5 mm	8.7500 mm	0.005
	Pro root	8 mm	9 mm	8.5000 mm	
	Ortho	8 mm	8 mm	7.3750 mm	
	Cem cement	7.5 mm	8 mm	7.8750 mm	
	Total	7 mm	9.5 mm	8.1250 mm	

Table 2 The diameter of the growth-inhibiting zone of microorganisms after 48 h

bacteria and material names		Minimum	Maximum	Average	*p*-value
Enterococcus faecalis	AGM	8 mm	8.5 mm	8.3750 mm	0.410
	Pro root	7 mm	9 mm	7.8750 mm	
	Ortho	8 mm	9 mm	8.3750 mm	
	Cem cement	8 mm	8 mm	8.0 mm	
	Total	7 mm	9 mm	8.1563 mm	
Escherichia coli	AGM	9 mm	9 mm	9.0 mm	0.049
	Pro root	7.5 mm	12 mm	10.6250 mm	
	Ortho	10 mm	12.5 mm	10.8750 mm	
	Cem cement	8 mm	9 mm	8.50 mm	
	Total	7.5 mm	12.5 mm	9.750 mm	
Candida albicans	AGM	8 mm	9.5 mm	8.750 mm	0.005
	Pro root	8 mm	9 mm	8.50 mm	
	Ortho	7 mm	8 mm	7.3750 mm	
	Cem cement	7.5 mm	8 mm	7.8750 mm	
	Total	7 mm	9.5 mm	8.1250 mm	

Then bioceramic materials AGM MTA, Ortho MTA, Pro root MTA, and Cem cement were placed in the wells using sterile carrier amalgam. All plates were kept at room temperature for two hours and then incubated at 37 °C for 24 h. Then, the diameter of the growth inhibitory zone was measured using a ruler with an accuracy of 0.5 mm [14]. After 48 and 72 h, the diameter of the growth inhibitory zone was measured again and the results of the data were compared and statistically analyzed [13]. All reagents were used for analytical grade. In each solution, pH was regulated by hydrochlorid acid or sodium hydroxide. Each experiment was conducted in triplicates. After collecting the information and entering the data into the computer using SPSS software version 22, the data was analyzed. Statistical analysis was performed using a one-way ANOVA for the mean zones of growth inhibition among the materials tested. The Post Hoc test was run for multiple comparisons. Statistically significant differences among the groups were set at $p<0.05$.

Results

The findings from Tables 1 and 2, and 3 showed that the types of bio-ceramic material studied against *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans* fungus showed antibacterial and antifungal effects in 24, 48, and 72 h by well method. In the context of the relationship between the type of microorganism and the diameter of the growth inhibitory zone after 24 h for each type of bio-ceramic material, there was no significant relationship only for *Enterococcus faecalis* and both Escherichia and Candida microorganisms had a significant relationship. In addition, the highest average diameter of the growth inhibitory zone related to Ortho MTA was against Escherichia coli, and the lowest average diameter of the growth inhibitory zone was related to Ortho MTA against Candida albicans (Table 1).

Table 3 The diameter of the growth inhibiting zone of microorganisms after 72 h

bacteria and material names		Minimum	Maximum	Average	*p*-value
Enterococcus faecalis	AGM	8 mm	8.5 mm	8.3750 mm	0.410
	Pro root	7 mm	9 mm	7.8750 mm	
	Ortho	8 mm	9 mm	8.3750 mm	
	Cem cement	8 mm	8 mm	8.0 mm	
	Total	7 mm	9 mm	8.1563 mm	
Escherichia coli	AGM	9 mm	9 mm	9.0 mm	0.049
	Pro root	7.5 mm	12 mm	10.6250 mm	
	Ortho	10 mm	12.5 mm	10.8750 mm	
	Cem cement	8 mm	9 mm	8.50 mm	
	Total	7.5 mm	12.5 mm	9.750 mm	
Candida albicans	AGM	8 mm	9.5 mm	8.750 mm	0.005
	Pro root	8 mm	9 mm	8.50 mm	
	Ortho	7 mm	8 mm	7.3750 mm	
	Cem cement	7.5 mm	8 mm	7.8750 mm	
	Total	7 mm	9.5 mm	8.1250 mm	

In the context of the relationship between the type of microorganism and the diameter of the growth inhibitory zone after 48 h for each type of bio-ceramic material, there was no significant relationship only for *Enterococcus faecalis* and both *Escherichia* and *Candida* microorganisms had a significant relationship. In addition, the highest average diameter of the growth inhibitory zone related to Ortho MTA was against *Escherichia coli* and the lowest average diameter of the growth inhibitory zone related to Ortho MTA was against *Candida albicans* (Table 2).

In the context of the relationship between the type of microorganism and the diameter of the growth inhibitory zone after 72 h for each type of bio-ceramic material, there was no significant relationship only for *Enterococcus faecalis*, and both *Escherichia* and *Candida* microorganisms had a significant relationship. In addition, the highest mean diameter of the growth inhibitory zone was related to Ortho MTA material against *Escherichia coli* and the lowest mean diameter of the growth inhibitory zone related to Ortho MTA material was against *Candida albicans* (Table 3).

Discussion

Various materials have been used to close the perforations, among which, a mineral trioxide aggregate (MTA) has good sealing power and tissue compatibility. This material is used in the treatment of open apex teeth, pulpotomy, pulp capping, root-end filling and perforation management, and pulp revascularization. Also, calcium-enriched mixed cement (Cem cement) contains calcium compounds. This material has good biocompatibility and can induce hard tissue, including hydroxyapatite [17]. The outstanding feature mentioned in the studies for this substance is its antibacterial and antifungal effects. For root-end filling materials, having antimicrobial properties is considered an advantage, and from this point of view, Cem cement is equal to calcium hydroxide, while it is significantly better than MTA [18]. One of the important factors involved in pulp and periapical diseases is microorganisms, which also play an important role in root canal treatment failure. In cases where there is no recovery after the usual root canal treatment and re-treatment is not possible, the therapist moves towards Epico surgery. Endodontic filling materials should be biocompatible, nonabsorbable, dimensionally stable, have antibacterial and antifungal activity, radiopaque, as well as a good apical flood to prevent the movement of bacteria and bacterial products from the root canal system to the tissue. prevent periapical [15]. *Enterococcus faecalis* bacteria are often isolated from infected root canals. Especially in resistant infections after root canal treatment, the presence of serine protease and collagen-binding protein (Ace) helps the adhesion of *Enterococcus faecalis* bacteria to the root canal and invasion of dentinal tubules [19].

McHugh et al. investigated the pH required to kill *Enterococcus faecalis* and found that the growth of *Enterococcus faecalis* was delayed at a pH of 10.5 to 11 and that the bacteria could not survive at a pH greater than 11.5 [20].

It can be assumed that *Enterococcus faecalis* will not survive in the vicinity of MTA due to high alkalinity (pH equal to 11–12), but in clinical conditions, due to the buffering capacity of dentin, the desired high pH cannot be maintained after using MTA. In addition, *Enterococcus faecalis* has a proton pump that helps reduce the intracellular pH level [21]. *Candida albicans* fungus is part of the natural flora of the oral cavity. This fungus is sometimes seen in early root canal infections but appears more common in the root canals of avulsed teeth where treatment has failed. *Albicans* can attack dentin tubules and have high resistance to some intracanal drugs such as calcium hydroxide. *Escherichia coli* is a gram-negative facultative anaerobic bacterium that is often found in infected root canals [14]. These microorganisms are found with greater frequency in cases of prolonged treatment, flare-ups, and failed treatments and can enter the root canal system before or after treatment and cause secondary infection [15]. According to the mentioned explanations, one of the important features of MTA is having antibacterial and antifungal properties. AGM MTA is a new type of Iranian MTA that has recently been released to the market, and so far no similar study has been conducted on this important property in this material, which is the strength of this study. In the present study, a non-growth aura was observed for all four types of bio-ceramic material against the mentioned microorganisms in the MHA culture medium. There was no significant difference in the *Enterococcus faecalis* bacteria plate, but there was a significant difference in the *Escherichia coli* and *Candida albicans* fungus plates. In a cross-sectional study which Moazami et al. conducted in Shiraz in 2020 evaluated the antibacterial effect of bio-ceramics against *Escherichia coli*, *Enterococcus feacalis* and antifungal effect against *Candida albicans*, and finally, they concluded that both Pro Root MTA and Nano-fast Cement had the same level of effect against oral microorganisms and did not differ significantly from each other [14].

In a cross-sectional study under the title of evaluating the antibacterial and antifungal properties of three fillers that Megan et al. conducted in India in 2016, during their study, they investigated the antibacterial effect of bio-ceramics against bacteria *Enterococcus faecalis*, *Staphylococcus aureus* and they were antifungal against *Candida albicans* fungus using agar diffusion method. Then they compared the results between three materials MTA, Biodentin, and RMGIC (Resin Modify Glass Ionomer Cement). Finally, they concluded that all three mentioned substances had antibacterial properties against the mentioned microorganisms. Also, RMGIC had no effect against *Candida albicans*, and among them, Biodentin had the most effect [15], which is consistent with the results of the present study. In a cross-sectional study under the title of evaluating the antibacterial and antifungal properties of calcium silicate fillers in Turkey in 2016, Ebru et al. investigated the antibacterial effect against *Enterococcus faecalis* bacteria and the antifungal effect against *Candida albicans* using the agar diffusion method. Then they compared the results between MTA Angelus, Biodentin, and DiaRoot Bioaggregate cements. The results showed that all three mentioned substances have antibacterial and antifungal properties and their spectra are slightly different from each other, but they are not significantly different [13], which is in line with the results of the present study. In a cross-sectional study titled the evaluation of the antimicrobial effect of MTA mixed with fluorohydroxyapatite on *Enterococcus faecalis* bacteria, conducted by Bolhari et al. in 2021 in Tehran, to investigate the evaluation of the antimicrobial effect of pure MTA, MTA mixed with 10% (W/V) of nano FHA (Fluor-hydroxyapatite) and MTA mixed with 15% (W/V) nano FHA. In the results of the antimicrobial test, the diffusion of agar disk showed the absence of growth-inhibiting areas in all samples after 24 h [22], which is inconsistent with the results of the present study. It seems that this difference was due to the difference in the method of doing the work and the type of cultivation environment.

In Khalid et al. study in 2006, aimed to investigate the antimicrobial effect of MTA against *Enterococcus faecalis* and *Streptococcus sanguinis* bacteria. The results of the study showed that GMTA (gray-colored MTA) has antibacterial activity against *Enterococcus faecalis* [23], which is consistent with the present study.

In a cross-sectional study titled in vitro evaluation of antibacterial properties of MTA against five oral bacteria conducted by Kim Ryan et al. in 2015 in South Korea. In the above study, they investigated the antibacterial effect of MTA-Angelus, Endocem MTA, and Pro Root MTA against *Streptococcus mutans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei*. Disc diffusion test showed that the antibacterial activity against *S. mutans*, *L. rhamnosus*, *L. paracasei*, and *P. gingivalis* ranked in decreasing order of MTA-Angelus > ProRoot MTA > Endocem MTA ($p < 0.05$) [19], which is inconsistent with the results of the present study. It seems that this difference is due to the difference in the method of doing the work, the type of culture medium, and the type of strain of *Enterococcus faecalis* (ATTC 4082). The antimicrobial properties are exerted during the setting process by increasing the pH and ion release from the material [24]. This study takes a critical look at the antimicrobial property of currently available bioceramic materials used in endodontics and looks forward to embracing possible new technologies that may facilitate the elimination of all microorganisms for long-term endodontic success. Bacteria have evolved various mechanisms to combat antimicrobial agents, including altering their effective concentration. Overall, the ability of bacteria to alter the effective concentration of antimicrobial agents poses a significant challenge in the treatment of infections. As bacteria continue to evolve and develop new mechanisms of resistance, it is essential for researchers and healthcare providers to stay vigilant and develop new strategies to combat antimicrobial resistance.

Conclusion

The results of this study showed that each of the bio-ceramic materials AGM, Pro root, Cem cement, and Ortho have antibacterial and antifungal effects. In the case of *Candida albicans* fungus, AGM MTA material and in the case of *Escherichia coli* bacteria, Ortho MTA material has the most effect, and it can be said that the above bio-ceramics are still recommended as a suitable material for filling the end of the root. Considering that few studies have been conducted in the field of investigating the antibacterial and antifungal activity of AGM MTA bio-ceramic material against common oral micro-organisms in Iran, more studies in this field with different methods and against more microorganisms, especially *actinomycetes*, have been conducted to accurately determine the superior material.

Abbreviations

MTA Mineral Trioxide Aggregate

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Author contributions

Conceptualization: Maryam Tabibi. Data curation: Maryam Tabibi. Formal analysis: Maryam Tabibi. Funding acquisition: Fatemeh Asadi Yalin, Maryam Tabibi. Investigation: Fatemeh Asadi Yalin, Maryam Tabibi. Methodology: Fatemeh Asadi Yalin, Maryam Tabibi, Mohammad Aghaali. Project administration: Maryam Tabibi, Alireza Majidi, Mohammad Aghaali. Resources: Maryam Tabibi. Software: Fatemeh Asadi Yalin, Maryam Tabibi, Alireza Majidi, Mohammad Aghaali. Supervision: Maryam Tabibi, Alireza Majidi, Mohammad Aghaali. Validation: Maryam Tabibi. Visualization: Maryam Tabibi. Writing–original draft: Faezeh Kabiri, Alireza Rasouli. Writing–review & editing: Faezeh Kabiri, Alireza Rasouli.

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Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files.

Declarations

Ethics approval and consent to participate

No human or animal samples were used in this article.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

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