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In vitro antimicrobial activity of mineral trioxide aggregate, Biodentine, and calcium-enriched mixture cement against *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans* using the agar diffusion technique

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ABSTRACT

Background: This study assessed the antimicrobial activity of Biodentine, mineral trioxide aggregate (MTA), and calcium-enriched mixture (CEM) cement against *Enterococcus faecalis*, *Streptococcus mutans* , and *Candida albicans*.

Materials and Methods: In this *in vitro* study, microbial suspensions were inoculated onto agar plates. The antimicrobial effects of MTA, Biodentine and CEM cement were assessed against *E. faecalis*, *S. mutans*, and *C. albicans* by the agar diffusion test. In each experimental group, 7 plates containing 3 wells were prepared and immediately filled with freshly mixed cements. Positive and negative control plates were prepared with/without the bacterial suspension, respectively. After 2 h of preincubation at room temperature, the plates were incubated at 37°C for 24 h. The diameter of growth inhibition zones was measured after 24 h. Data were analyzed using ANOVA and Tukey's test (α = 0.05).

Results: Biodentine showed strong antimicrobial activity against all three microorganisms with an average inhibition zone of 9.10 mm. The inhibitory effect of Biodentine on *E. faecalis* and *C. albicans* was significantly superior to that of the other two cements ($P < 0.05$). MTA and CEM cement showed significantly higher antimicrobial activity against *S. mutans* ($P < 0.05$). The antimicrobial effects of Biodentine on *S. mutans* and *E. faecalis* were significantly greater than on *C. albicans* ($P < 0.05$).

Conclusion: All cements revealed antimicrobial properties against the tested microbial strains. Biodentine had stronger antimicrobial effects against *E. faecalis* and *C. albicans* compared with MTA and CEM cement. Furthermore, the largest inhibition zones around all three cements belonged to *S. mutans*.

Key Words: Biodentine, calcium-enriched mixture cement, *Candida albicans*, *Enterococcus faecalis*, mineral trioxide aggregate

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INTRODUCTION

Microorganisms are the main culprits responsible for the development of pulpal and periapical diseases and

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can cause endodontic treatment failures.[1] Provision of an effective seal to prevent recontamination as well as successful elimination or reduction of microorganisms in the root canal system can positively affect the outcome of endodontic treatment.[2] The endodontic procedural steps such as chemomechanical instrumentation, root canal irrigation, application of intracanal medicaments, and sealing of the pulp chamber eliminate the microorganisms and enhance regeneration of periapical tissues.[3] After chemomechanical preparation, however, some bacteria may remain in ramifications and dentinal tubules and lead to treatment failure. There is no definite strategy to completely eliminate residual bacteria.[4] Moreover, many of the currently available biomaterials may not create an ideal seal. Thus, it is imperative to use endodontic cements with the ability to inhibit bacterial growth.[2] Therefore, the antimicrobial properties of these biomaterials should be investigated. The agar diffusion test is the most widely used technique to assess the antimicrobial properties of dental materials.[5]

Mineral trioxide aggregate (MTA) was first introduced by Torabinejad *et al*. Its application has been successful for furcal and lateral root surface perforation repairs, as well as root-end filling, vital pulp capping, and also as an apical plug in apexification.[6] MTA is supplied in the form of a powder with fine hydrophilic particles that form a colloidal gel in the presence of water. It is solidified and forms hard cement within nearly 4 h.[7] MTA has many advantages such as osteogenic and regeneration potential, good marginal sealability, bioactivity, biocompatibility, and antibacterial effects.[5,8] However, long setting time, high cost, poor handling properties, and tooth discoloration are its main drawbacks.[9-11]

Calcium-enriched mixture (CEM) cement was introduced in 2006 and contains different calcium compounds.[12] The mixed CEM cement consists of water-soluble calcium and phosphate, which immediately lead to the formation of hydroxyapatite during and after the setting period.[13] This cement is biocompatible and its clinical application is similar to that of MTA; however, their chemical compositions are different. This cement creates an effective seal and provokes the healing of hard tissue as does the MTA.[13-15] However, in comparison with MTA, it has a shorter setting time, lower cost, lower tooth discoloration potential and easier handling.[16-18] Moreover, CEM cement potentially prevents bacterial growth.[19-21]

Biodentine is a novel calcium-silicate based endodontic cement. Its powder mainly consists of tricalcium and dicalcium silicate, calcium carbonate and zirconium dioxide as a contrast medium. The liquid contains calcium chloride, which accelerates the setting reactions and is used as a water-reducing agent in aqueous solutions with a mixture of polycarboxylate and sets in 12 min.[22-24] Biodentine is a dentine replacement material, which can be used for filling of deep and extensive coronal caries, restoring of deep cervical and radicular lesions, pulpotomy and pulp capping, furcal and root perforation repair, management of internal and external resorptions, apexification, and surgical root-end filling.[25,26]

A number of studies have investigated the antimicrobial effects of MTA, Biodentine and CEM cement. In a study by Bhavana *et al*., in 2015, Biodentine showed stronger inhibitory effect than MTA on *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*.[27] Moreover, Asgary *et al*., in 2007, reported that antibacterial properties of CEM cement against *E. faecalis* were higher than those of MTA.[28] According to Koruyucu *et al*., in 2015, Biodentine and MTA showed similar antibacterial effects against *E. faecalis*.[29]

The aim of this experimental study was to compare the antimicrobial activity of MTA, Biodentine and CEM cement against *E. faecalis*, *S. mutans* and *C. albicans* by the agar diffusion technique.

MATERIALS AND METHODS

In this *in vitro* experimental study, the MTA (Angelus, Brazil), Biodentine (Septodont, France) and CEM cement (Yektazist, Iran) were individually mixed according to the manufacturers' instructions. The antimicrobial effects of these endodontic cements were evaluated against three reference strains namely *E. faecalis* (ATCC 29212), *S. mutans* (ATCC 35668), and *C. albicans* (ATCC 10231) by the agar diffusion method. The strains were obtained from the Department of Microbiology, Faculty of Dentistry, Azad University, Isfahan (Khorasgan) branch. Trypticase soy broth (Merck, Germany) and Sabouraud dextrose broth (Merck, Germany) were used for activation of microorganisms. Then, the overnight cultures of microorganisms were diluted by sterile trypticase soy broth and Sabouraud dextrose broth to obtain a suspension with 0.5

McFarland standard turbidity, which corresponds to a concentration of 1.5×10^8 CFUs/mL. All microbial strains were confirmed with growth characteristics and Gram-staining.

Standard suspensions of *E. faecalis* and *C. albicans* were inoculated onto Mueller-Hinton agar plates (Merck, Germany), and *S. mutans* suspension was inoculated onto blood agar plates (Merck, Germany) with sterile cotton swabs using the lawn culture method. Three wells, 4 mm deep and 5 mm in diameter, were prepared on each plate with a sterile Pasteur pipette, and immediately filled with freshly mixed test materials. For prediffusion of the materials, the plates were placed at room temperature for 2 h. Then, all the plates were incubated at 37°C and assessed after 24 h.[19,27]

The total number of plates, including the test plates and positive and negative controls, was 26. The plates were randomly divided into three groups for each microorganism. In each group, 7 plates containing three wells for testing each of the three cements were considered. The antimicrobial effect of each cement was tested seven times against each microorganism. Positive and negative control plates were prepared with and without the bacterial suspension, respectively and kept for the same incubation time under similar conditions. All experiments were performed in sterile conditions.

The diameter of microbial growth inhibition zones was measured by a digital caliper with 0.01 mm accuracy by an independent observer. Data were analyzed using SPSS software version 22 (SPSS Inc., Chicago, IL, USA), and the results were expressed as means and standard deviations. To compare the differences between the materials, data were analyzed statistically by one-way ANOVA and Tukey's *post hoc* test for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

The positive control groups showed microbial growth, while there was no sign of microbial growth in the negative control groups. Growth of all microbial species was inhibited by all the tested materials. Table 1 shows the antimicrobial activity of the cements against all tested microorganisms, which was evaluated by determining the mean and standard deviation of growth inhibition zones (mm) after 24 h. The greatest antimicrobial effect belonged to Biodentine and CEM cement against *S. mutans* and the least antimicrobial effect belonged to CEM cement against *E. faecalis*.

The maximum mean diameter of growth inhibition zone of *E. faecalis* and *C. albicans* was found around Biodentine, while the growth inhibition zones around MTA and CEM cement were smaller.

Tables 2-4 show pairwise comparisons of the cements against the three microorganisms, done by the Tukey's test. The results showed that the antimicrobial activity of Biodentine against *E. faecalis* was significantly higher than that of MTA and CEM cement ($P < 0.001$). Moreover, the antifungal activity of Biodentine against *C. albicans* was significantly greater than that of MTA ($P < 0.001$) and CEM cement ($P = 0.002$). In this study, the antimicrobial effect of CEM cement against *S. mutans* was significantly higher than that of MTA ($P = 0.038$), while there was no significant difference between Biodentine and CEM cement ($P = 0.958$). Although the antimicrobial effect of Biodentine and CEM cement against *S. mutans* was superior to the effect of MTA, the difference between Biodentine and MTA was close to the significant level ($P = 0.066$). Furthermore, for all three cements, the maximum mean growth inhibition zone belonged to *S. mutans*.

DISCUSSION

An ideal endodontic cement should have bacteriostatic or bactericidal properties. Furthermore, root-end filling biomaterials should inherently have antimicrobial effects because many bacteria causing primary infections or treatment-resistant microorganisms may remain in the root canal system in case of persistent endodontic infections.[30] In chronic or refractory periapical lesions, facultative bacteria, and yeasts are the most prevalent and predominant microorganisms.[27]

Table 1: Antimicrobial activity of the cements against the three microbial species

Materials	Microorganisms		
	Enterococcus faecalis (mm)	*Streptococcus mutans* (mm)	*Candida albicans* (mm)
Biodentine	9.46 (1.064)*	9.67 (0.963)	8.17 (0.515)
MTA	6.73 (0.696)	8.57 (0.884)	6.73 (0.408)
CEM cement	6.54 (0.623)	9.79 (0.691)	7.11 (0.515)

*Mean (SD). The growth inhibition zones are presented in millimeters. SD: Standard deviation; MTA: Mineral trioxide aggregate; CEM: Calcium-enriched mixture

Table 2: Pairwise comparisons of cements against *Enterococcus faecalis*

Material (I)	Material (J)	Mean difference (I-J) (mm)	SE	Significance level	95% CI	
					Lower bound	Upper bound
Biodentine	MTA	2.729*	0.437	0.000	1.61	3.84
	CEM	2.920*	0.437	0.000	1.81	4.03
MTA	Biodentine	-2.729*	0.437	0.000	-3.84	-1.61
	CEM	0.191	0.437	0.900	-0.92	1.31
CEM	Biodentine	-2.920*	0.437	0.000	-4.03	-1.81
	MTA	-0.191	0.437	0.900	-1.31	0.92

*The mean difference is significant at the 0.05 level. CI: Confidence interval; SE: Standard error; MTA: Mineral trioxide aggregate; CEM: Calcium-enriched mixture

Table 3: Pairwise comparisons of cements against *Streptococcus mutans*

Material (I)	Material (J)	Mean difference (I-J) (mm)	SE	Significance level	95% CI	
					Lower bound	Upper bound
Biodentine	MTA	1.099	0.456	0.066	-0.07	2.26
	CEM	-0.127	0.456	0.958	-1.29	1.04
MTA	Biodentine	-1.099	0.456	0.066	-2.26	0.07
	CEM	-1.226*	0.456	0.038	-2.39	-0.06
CEM	Biodentine	0.127	0.456	0.958	-1.04	1.29
	MTA	1.226*	0.456	0.038	0.06	2.39

*The mean difference is significant at the 0.05 level. CI: Confidence interval; SE: Standard error; MTA: Mineral trioxide aggregate; CEM: Calcium-enriched mixture

Table 4: Pairwise comparisons of cements against *Candida albicans*

Material (I)	Material (J)	Mean difference (I-J) (mm)	SE	Significance level	95% CI	
					Lower bound	Upper bound
Biodentine	MTA	1.440*	0.258	0.000	0.78	2.10
	CEM	1.059*	0.258	0.002	0.40	1.72
MTA	Biodentine	-1.440*	0.258	0.000	-2.10	-0.78
	CEM	-0.381	0.258	0.323	-1.04	0.28
CEM	Biodentine	-1.059*	0.258	0.002	-1.72	-0.40
	MTA	0.381	0.258	0.323	-0.28	1.04

*The mean difference is significant at the 0.05 level. CI: Confidence interval; SE: Standard error; MTA: Mineral trioxide aggregate; CEM: Calcium-enriched mixture

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. It has been isolated from 22% to 77% of teeth with failed endodontic treatments.[31] *E. faecalis* is resistant to high pH and has the ability to invade the dentinal tubules. Thus, it is highly resistant to intracanal medicaments.[32,33] *S. mutans* can have significant effects on both the initial and secondary pulpal lesions.[34] *C. albicans* is capable of creating a biofilm on various surfaces and may be found in cases of persistent and secondary infections.[35] For the aforementioned reasons, the antimicrobial activity of the three abovementioned microorganisms was tested against three commonly used endodontic cements in this study.

The agar diffusion test, which is the most common method for the assessment of antimicrobial activity, was used in this study.[36] The diffusion capability of the material through the medium greatly affects the outcome of the agar diffusion test.[37] However, the results of agar diffusion test also depend on many factors, such as the selection of microorganisms and the agar medium used, standardization of the concentration of microbial suspensions, incubation times and measurement of growth inhibition zones.[38] The incubation period was considered 24 h in this study; also, the microbial suspension was diluted to 0.5 McFarland standard concentration, which was similar to the previous studies.[19,27] Moreover, according to similar previous studies, all plates were maintained at room temperature for 2 h for prediffusion of materials.[19,27] In this study, attempts were made to standardize the testing conditions to minimize the effect of confounders.

In this study, the antimicrobial properties of Biodentine, MTA and CEM cement were assessed,

and freshly mixed materials were transferred into agar plates immediately because the antimicrobial efficacy of materials is affected by their degree of polymerization.[5] Our results showed that all the test materials had antimicrobial effects against the tested microorganisms. The antimicrobial characteristics of MTA may be due to its alkalinity.[39] A pH higher than nine can reversibly or irreversibly deactivate cellular membrane enzymes of the microorganisms, leading to loss of their biological activity.[40] The initial pH of the freshly mixed MTA is 10.2, but it reaches 12.5 after 3 h, probably due to the release of calcium hydroxide during the hydration process.[41]

The main constituents of CEM cement include alkaline earth metal hydroxides and oxides (e.g., calcium hydroxide and calcium oxide), calcium silicate, and calcium phosphate. The pH of CEM cement is around 10.71 after 1 h.[42] During and after mixing with its liquid, the hydration reactions occur, which produce calcium hydroxide. When CEM cement is transferred into agar plates and contacts the medium, calcium hydroxide dissociates into calcium and hydroxyl ions, which raise the pH and calcium levels.[28] Calcium hydroxide with a pH of 12.5 is a major CEM cement byproduct.[7,43,44] This can explain the optimal antimicrobial activity of this cement, at least in part. Another possible reason can be the superior diffusion properties of antimicrobial constituents of CEM cement.[28]

Biodentine has greater mechanical properties and less solubility. It provides a tighter seal, has more convenient handling properties and requires less time to set in comparison with other materials such as MTA.[22] The findings of the present study showed that Biodentine had better antifungal and antibacterial effects than MTA. Antimicrobial properties of Biodentine are strongly related to its calcium release and alkalinity. During the hydration process of the cement, colloidal gel is formed, which leads to the release of calcium hydroxide that prevents bacterial growth. Furthermore, the pH of Biodentine increases to 12.5 during setting; therefore, bacterial growth is inhibited, and the surrounding areas are disinfected.[45]

None of the previous studies has compared the antimicrobial activity of MTA, Biodentine and CEM cement. Unlike this study, Estrela *et al*. demonstrated that MTA did not show any antimicrobial activity against *C. albicans* and *E. faecalis*.[46] Moreover, Torabinejad *et al*. revealed that MTA was not effective against *E. faecalis*,[2] which contradicted the results of this study. Our results revealed that Biodentine had greater antimicrobial efficacy against *C. albicans* and *E. faecalis* than the other two cements. Chopra *et al*. indicated that the growth inhibition zones of these two microorganisms around Biodentine were significantly larger than the area around MTA,[45] which was consistent with the results of this study. Furthermore, Jose *et al*., demonstrated that Biodentine had significantly superior antimicrobial activity compared with MTA,[30] which was confirmed by the results of the present study. Similar to the present study, Bhavana *et al*. concluded that antimicrobial activity of Biodentine against *E. faecalis*, *S. mutans* and *C. albicans* was greater than that of MTA. In addition, in the study by Bhavana, the inhibition zone diameters of *S. mutans* around Biodentine and MTA were significantly greater than around *E. faecalis* and *C. albicans*,[27] which is parallel to the results of the current study on MTA. Nourzadeh *et al*., in 2019, discussed that CEM cement had a higher inhibitory effect on *E. faecalis* than Biodentine,[47] while the present study suggested a stronger antibacterial activity than CEM cement against *E. faecalis*. It should be mentioned that the discrepancy between the results of studies can be attributed to the available nutrients, incubation period, oxygen pressure level, techniques of assessment, and diverse laboratory set-ups used.[27]

CONCLUSION

1. All three cements had growth inhibitory effects on the three types of microorganisms tested
2. Compared with MTA, Biodentine showed greater inhibitory effects against *E. faecalis* and *C. albicans*, which are resistant microorganisms in endodontic treatment
3. The greatest inhibition zone in all three materials was related to *S. mutans*.

Biodentine with its potent antimicrobial effect can be considered as an appropriate alternative to MTA and CEM cement in endodontic treatment. Due to the lack of studies to compare the antimicrobial properties of Biodentine, MTA and CEM cement, further studies are recommended before introducing Biodentine as an ideal material for endodontic treatments.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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