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Antibacterial behavior of calcium fluoride-modified tricalcium silicate cement against different oral bacterial strains

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Aim: This study aimed to evaluate the effect of adding CaF_2 to Biodentine on its antibacterial behavior against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* strains and the fluoride release.
Materials and methods: The study included three main groups: unmodified Biodentine, 5% wt CaF_2-modified Biodentine, and 10% wt CaF_2-modified Biodentine. The antibacterial activity of the tested cement was evaluated against the three bacterial strains through direct contact and agar diffusion tests. Fluoride release in deionized water at different intervals (1 day, one week, and one month) was assessed using ICP-MS.
Results: The direct contact test revealed that the different experimental biodentine groups had lower optical density than the bacteria groups after one day, which was confirmed at seven days. The 10% group showed a significantly higher antibacterial effect against *S. mutans* and *L. acidophilus* than the other biodentine groups. The agar diffusion test showed significant differences between groups regarding *Streptococcus mutans* and *Lactobacillus acidophilus*. The positive control group had significantly higher inhibition zone values than the 5% CaF_2-modified and unmodified Biodentine groups. The 10% CaF_2-modified group showed no statistically significant difference compared to the positive control. For *Enterococcus faecalis*, the positive control exhibited a significantly higher value than other groups. None of the experimental groups showed inhibition zones against *Enterococcus faecalis*.
Conclusions: Adding 10% CaF_2 to Biodentine could improve its antibacterial effect against S. mutans and L. acidophilus. However, its effectiveness against *Enterococcus faecalis* is questionable. The addition of 10% CaF2 also offers fluoride-releasing qualities to Biodentine.

Keywords: Biodentine, Tricalcium silicate cement, Antibacterial, Calcium fluoride, Anticariogenic.

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Introduction

Various restorative and endodontic clinical procedures can significantly improve oral health. However, none can ensure instant and radical biofilm elimination or the prevention of recurrent infection. Therefore, the long-term clinical success of these treatments may depend on the antibacterial properties of the different materials used.[1-3]

Dental cements are cornerstone materials used in different clinical procedures. Among the ideal requirements for a successful dental cement are being biocompatible, possessing an antibacterial effect, having an acceptable sealing ability, and stimulating hard tissue regeneration. Finding the ideal material that fulfills all the requirements remains a challenge.[4]

Recent conservative guidelines leave more affected tissue with more residual bacteria.[3] These facts spotlight the need to develop antibacterial dental cement. Such a material could theoretically reduce the risk of clinical failure, allow more conservation of the tooth structure, decrease the extent of microleakage, and help in maintaining healthy tissues.[5]

Tricalcium silicate cement has various dental applications in restorative dentistry, including vital pulp therapy, apexification, root-end filling in endosurgery, and perforation repair, and some products like Biodentine are used as dentine substitute restorative material.[6,7]

Biodentine, launched in 2011, is a fast-setting bioactive dentine replacement. Its powder comprises highly purified tricalcium silicate, dicalcium silicate, calcium carbonate, and zirconia radiopacifier. The mixing liquid is water with a calcium chloride setting accelerator and a hydro-soluble polymer water-reducing agent.[8] Biodentine has antibacterial potential, attributed to the release of calcium hydroxide due to the hydration reaction, which aids in the disinfection of dentin and the inhibition of various microorganisms' growth.[9]

The anti-cariogenic effects of fluoride are well-documented and rely on various mechanisms, such as reducing demineralization, enhancing remineralization, plaque formation interference, and bacterial growth and metabolism inhibition. All these mechanisms are assumed to possess the anti-cariogenic effect of the fluoride-releasing restorative materials.[10,11]

Fluorides exhibit antibacterial behavior through various mechanisms that affect bacterial metabolism. Fluoride acts as a quasi-irreversible inhibitor of glycolytic enzymes like enolase. Although glycolytic pathways exist in most living systems, the sensitivity to fluoride differs significantly between microorganisms and eukaryotic systems.[12]

Several studies have demonstrated the demineralization inhibition effect of fluoride on dental hard tissues. These studies showed that fluoride could be incorporated into the enamel, forming more acid-resistant fluorapatite crystals.[13] Various forms of fluoride have been added to the formulation of various materials, such as fluoroaluminosilicate glasses, calcium fluoride, stannous fluoride, sodium fluoride, organic amine fluorides, and ytterbium fluoride.[14]

Streptococcus mutans is one of the well-known oral flora that have a significant contribution to dental caries. Its colonization of tooth structure is highly correlated to the early stages of caries development. Lactobacillus species were commonly found in deep dentin carious lesions. With the increase in caries progression, a significant increase in Lactobacillus occurs.[15] E. faecalis is a highly resistant strain to various antibacterial formulations.[16] Furthermore, E. faecalis can endure challenging

environments with high alkalinity and a limited nutrient supply. E. faecalis can grow as a biofilm on radicular dentine walls and in treated canals, even without synergistic effects from other bacterial strains. Therefore, it is considered an essential biological indicator for endodontic infection.[17,18]

Calcium fluoride has been found to possess potent antibacterial activity against Streptococcus mutans. However, its antibacterial efficiency against other common human pathogens remains questionable.[12] Additionally, calcium fluoride may improve the bioactivity of Biodentine by enhancing remineralization with the formation of fluoroapatite [19,20]

Biodentine possesses a considerable in-vitro antibacterial performance against oral bacteria; however, its sustainability is still controversial. Additionally, some studies added antimicrobials such as titanium tetrafluoride (TiF_4), cetrimide, or chlorhexidine to Biodentine, leading to improved antimicrobial efficiency of the cement.[21] Reviews by Ruiz-Linares, Fagundes et al.[22] revealed limitations in studies that determine the antimicrobial efficacy of Biodentine, and their results are controversial. Therefore, improving the antibacterial behavior through a combination of calcium fluoride and tricalcium silicate-based cement could offer great benefits.[23]

Accordingly, this study aimed to assess the effect of the addition of calcium fluoride on the antibacterial properties of Biodentine against *S. mutans*, *L. acidophilus*, and *E. faecalis* bacterial strains, as well as the fluoride release at different time intervals.

Materials and methods

Experimental design and specimen's grouping

The study design included three main groups according to the tested cement, which are the unmodified Biodentine, the Biodentine modified by incorporating 5% wt. anhydrous CaF_2, and the Biodentine modified by incorporating 10% wt. anhydrous CaF_2. The different experimental groups were evaluated regarding antibacterial activity against three American Type Culture Collection (ATCC) strain microorganisms: *Streptococcus mutans*, *Lactobacillus acidophilus,* and *Enterococcus faecalis*.

Sample size calculation

A power analysis was performed to ensure adequate power to apply a statistical test of the null hypothesis that there would be no difference between different groups regarding the antibacterial activity. By adopting a power of 95%, alpha (α) and beta (β) levels set at 0.05, and an effect size (f) of 7.97 based on a previous study,[21] the minimum total required sample size was found to be eight samples (i.e., two samples per group). The sample size in the current study was increased to 7 samples for each group to account for possible failures during testing. The sample size was calculated using R statistical analysis software version 4.3.2 for Windows.

Cement preparation:

The Biodentine™ (Septodont, France. Lot number: B22105) was modified by incorporating 5% wt. (0.035 gm) and 10% wt. (0.07 gm) anhydrous 99.9% CaF_2 (Sigma Aldrich, Darmstadt, Germany. Lot number: MKBX9719V) powder into the cement powder capsule. Mechanical mixing was performed for 30 seconds using an amalgamator (3M Capmix™, Germany) to obtain a homogenous distribution of the CaF_2 within the powder. Then, five drops of the liquid were added to the Biodentine capsule and mixed for 30 seconds at 4650 rpm using an amalgamator according to the manufacturer's instructions.

Supply and preparation of microorganisms

Our study was conducted at the Microbiology Department, Faculty of Medicine, Ain Shams University, with ethical approval (FDASU-Rec ER122338) from the university's ethical committee. In this study, standard strains of Enterococcus faecalis (ATCC 29212), Streptococcus mutans (ATCC 25175), and Lactobacillus acidophilus (ATCC 4356) were used. Strains were homogenized with 5 milliliters of physiological saline. Brain–heart infusion (BHI) broth media was used for E. faecalis and S. mutans, while Man, Ragosa, and Sharpe (MRS) broth media were used with the L. acidophilus strain. The cultures were incubated for 48 hours at 37°C with CO_2 in an incubator (Heraeus, Germany). After 48 hours, the bacteria's growth was verified by gram stain and transferred to BHI agar or MRS broth agar solid media. Then, they were kept in an incubator with CO_2 at 37°C for 24 hours.[24]

I-Direct contact test

A direct contact test was performed to measure the antibacterial behavior against different bacterial strains quantitatively. The test relies on turbidimetric verification of bacterial growth in 96-well microtiter plates. Three plates were used to have separate plates for each bacterial strain. Freshly mixed cement from different experimental groups was coated on the sidewalls of the wells. Subsequently, a ten-microliter bacterial suspension (approximately 10^6 bacteria) was loaded on the cement. Cement specimens were carefully loaded on the side wall without any leakage toward the bottom of the well to prevent light interference and false readings in the spectrophotometer reader. After incubation for 1 hour at 37°C in a humid atmosphere, evaporation of the suspension's liquid was performed to provide direct contact between the bacteria and the tested cement surface. For wells containing S. mutans and E. faecalis, 250 µL BHI broth was added, while 250 µL MRS broth was added for L. acidophilus wells. Triplicate measurements for optical density were performed using an ELISA reader spectrophotometer (Thermo Lab Systems, Multiskan, Finland) at 600 nm. Measurements were performed immediately as a baseline, after one day, and after seven days to confirm the bacterial inhibition effect. The data were reported in optical density units (OD).[25]

II-Agar diffusion test

The antimicrobial effects of the different experimental groups were tested using a modified agar diffusion method based on Esteki et al. [26]. Well-grown bacterial colonies were loaded with sterile swabs after incubation. Bacterial suspensions were prepared in 3 milliliters of PSS with 0.5 McFarland turbidity (1.5×10^8 CFU/mL). E. faecalis and S. mutans strains were dispersed on all surfaces of BHI solidified agar plates, while L. acidophilus was dispersed on MRS broth solidified agar plates using sterile swabs. These solutions were mixed with a vortex mixer.

Twenty-one plates were prepared and randomly divided into three groups (n = 7) for each microorganism. Five wells (4 mm height X 5 mm diameter) were punched in each agar plate with a sterile pipette. Freshly mixed cement was immediately filled in 3 wells; the fourth was filled with the antibiotic disc (Amoxicillin antimicrobial susceptibility disc Thermoscientific™) as a positive control, and the fifth was filled with sterile filter paper as a negative control. Positive and negative controls were used to control the plate's quality and compare the experimental groups' inhibition zones against a known antibiotic. The plates were placed at room temperature for 2 hours to pre-diffuse the materials. Subsequently, all

the plates were incubated at 37°C and assessed after 24 hours. The diameter of microbial growth inhibition zones was measured using a digital caliper (Mitotoya, Japan) with 0.01 mm accuracy.

III. Fluoride ion release testing

Fourteen cement specimens (4 mm diameter X 6 mm height) were prepared from the CaF_2 –modified experimental groups (n = 7) using a split Teflon mold. Immediately after setting, specimens were stored in polypropylene sealed tubes at 37° C in 10 ml of deionized water (Water HPLC grade, Chemlab, Belgium). After different intervals (1 day, 1 week, and 1 month), specimens were transferred to a fresh 10 ml solution. The fluoride ion release of the tested materials at the predetermined time intervals (n = 7) was measured using inductively coupled plasma-mass spectrometry (ICP-MS AGILENT 8800, USA) at predefined time intervals.[27,28]

Statistical analysis

The numerical data were presented as mean and standard deviation (SD) values. The normality of the data was assessed using Shapiro-Wilk's test. Data were non-parametric and were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction for intergroup comparisons and Freidman's test followed by the Nemenyi post hoc test for intragroup comparisons. A repeated measure ANOVA test was employed for the fluoride release test. The significance level was set at $p < 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows.

Results

I-Direct contact test results (Optical density)

Table 1 and Figure 1 illustrate the direct contact test results. Baseline optical density (OD) values revealed no statistically significant difference among all groups with the different bacterial strains.

Across all the tested bacterial strains, the different experimental groups showed a significantly different optical density ($p < 0.001$). Post-hoc pairwise comparison showed that the different experimental Biodentine groups had a lower optical density than the bacteria groups after 1 day and were confirmed for 7 days, with no statistically significant difference between the 1 and 7 days.

The 10% CaF_2 group showed significantly better bacterial inhibition than other biodentine groups against Streptococcus mutans and Lactobacillus acidophilus. At the same time, there was no significant difference in bacterial inhibition between the 5% CaF_2 group and the unmodified biodentine group. Regarding the Enterococcus faecalis strain, the different biodentine groups showed bacterial inhibition with no statistical significance between the different experimental groups over one week. By observing the values of the bacterial group, the baseline values are less than one day and seven days in all bacterial strains, confirming the bacterial growth.

II- Agar diffusion test results (Bacterial inhibition zones)

Results comparing different groups using bacterial inhibition zone values are presented in Table 2.

There was a significant difference between the groups for S. mutans and L. acidophilus bacterial strains ($p < 0.001$). Post hoc pairwise comparisons revealed that the positive control group had a significantly higher value of inhibition zones than 5% and the unmodified Biodentine ($p < 0.001$). The 10% groups showed no statistically significant difference in the inhibition zone values compared to the antibiotic-positive control.

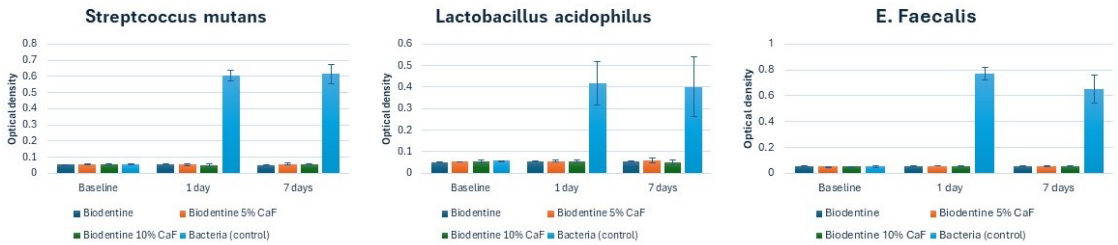

Figure 1: Bar charts showing the pattern of direct contact test (optical density) results of the different experimental groups against different bacterial strains.

Table (1): Means and standard deviations for the optical density values in the direct contact test of the different experimental groups against the different bacterial strains.

Bacteria	*Interval*	*Optical density (Mean±SD)*				*p-value*
		Biodentine	*Biodentine5% CaF_2*	*Biodentine 10% CaF_2*	*Bacteria*	
S. mutans	*Baseline*	0.055±0.001^{Aa}	0.055±0.004^{Aa}	0.054±0.002^{Aa}	0.054±0.003^{Ab}	**>0.05**
	1 day	0.058±0.004^{Ba}	0.057±0.005^{Ba}	0.051±0.010^{Ca}	0.604±0.031^{Aa}	**<0.001***
	7 days	0.059±0.001^{Ba}	0.058±0.005^{Ba}	0.052±0.003^{Ca}	0.615±0.060^{Aa}	**<0.001***
	p-value	**>0.05**	**>0.05**	**>0.05**	**<0.001***	
L.acidophilus	*Baseline*	0.056±0.002^{Aa}	0.056±0.000^{Aa}	0.055±0.006^{Aa}	0.055±0.001^{Ab}	**>0.05**
	1 day	0.057±0.003^{Ba}	0.058±0.004^{Ba}	0.051±0.004^{Ca}	0.417±0.101^{Aa}	**<0.001***
	7 days	0.058±0.003^{Ba}	0.059±0.011^{Ba}	0.050±0.010^{Ca}	0.402±0.137^{Aa}	**<0.001***
	p-value	**>0.05**	**>0.05**	**>0.05**	**<0.001***	
E.faecalis	*Baseline*	0.054±0.002^{Aa}	0.052±0.002^{Aa}	0.052±0.001^{Aa}	0.051±0.006^{Ab}	**>0.05**
	1 day	0.056±0.003^{Ba}	0.056±0.000^{Ba}	0.056±0.001^{Ba}	0.772±0.051^{Aa}	**<0.001***
	7 days	0.054±0.004^{Ba}	0.054±0.005^{Ba}	0.056±0.004^{Ba}	0.655±0.108^{Aa}	**<0.001***
	p-value	**>0.05**	**>0.05**	**>0.05**	**<0.001***	

For the E. faecalis bacterial strain, there was a significant difference between different groups ($p < 0.001$). Post hoc pairwise comparisons showed positive control to have significantly higher values than other groups ($p < 0.001$), while all the experimental groups showed no inhibition zones for E. faecalis.

Table (2): Means and standard deviations of bacterial inhibition zones (mm)in agar diffusion test of the different experimental groups against the different bacterial strains.

Bacteria	*Bacterial inhibition zones (mm) (Mean±SD)*				*p-value*
	Biodentine	*Biodentine 5%CaF_2*	*Biodentine 10%CaF_2*	*Antibiotic (positive control)*	
S. mutans	1.42 ± 0.05^{B}	1.44 ± 0.02^{B}	1.46 ± 0.21^{AB}	1.92 ± 0.11^{A}	**<0.05***
L.acidophilus	1.66 ± 0.09^{B}	1.66 ± 0.11^{B}	1.72 ± 0.08^{AB}	1.96 ± 0.11^{A}	**<0.05***
E.faecalis	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	1.96 ± 0.21^{A}	**<0.001***

III- Fluoride ion release results

The pattern of fluoride release in the different experimental groups with different time intervals is presented in Figure 2.

The 5% group showed a significantly increasing pattern of fluoride release from 1 day to 1 week, followed by a plateau. In contrast, the 10% group revealed a stable, high amount of fluoride release from 1 day to 1 month. The 10% group showed significantly higher fluoride release at all time intervals than the 5% group.

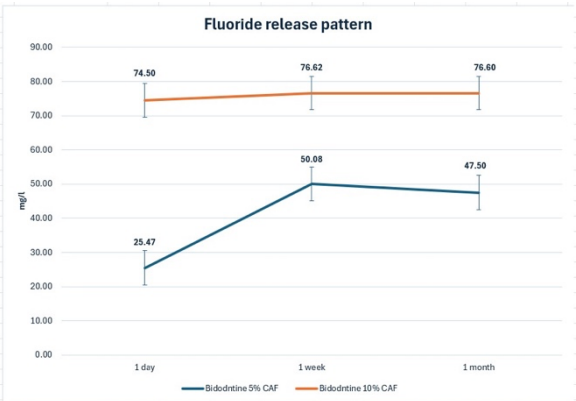

Figure 2: Line chart showing the fluoride release (mf/L) in CaF_2-modified groups at different time intervals

Discussion

Biodentine™ is recognized as the first fast-setting, high-strength tricalcium silicate-based cement for both endodontic and restorative applications.[29] The bioactive, remineralizing, and antibacterial restorative material like Biodentine™ may be advantageous in increasing more conservative restorative techniques' success rates.[30]

The formation of calcium fluoride deposits after the dental care procedure is limited by the low concentration of free calcium ions. The in-vitro synthesized calcium fluoride particles could overcome this problem.[14] Bala et al.[12] reported that calcium fluoride is a safe, encouraging antibacterial agent against microbial pathogens even at deficient concentrations.

Thus, it was of encouraging concern to assess the effects of adding CaF_2 to Biodentine™. The combination of both fluoride and tricalcium silicates may offer significant benefits for both endodontic and restorative dentistry. Based on previous studies[31,32] in addition to pilot data, Biodentine was modified with 5% and 10% by weight CaF_2.

Although Biodentine™ has demonstrated an antibacterial potential against certain bacterial strains, the data concerning its antibacterial effect is still conflicting.[22] This study assessed different bacterial strains representing coronal dentine caries bacteria (*Streptococcus mutans* and *Lactobacillus acidophilus*) and resistant endodontic infection (*Enterococcus faecalis*).

The agar diffusion test is considered one of the most common methods to evaluate the antibacterial properties of dental materials due to its simplicity and availability. However, its results showed significant variability when comparing the

antibacterial effects of different materials against different bacterial strains. This variability is due to the considerable influence of diffusion and solubility of the tested material in the agar on the antibacterial inhibition zone results.[33]

The direct contact test is a quantitative test used to measure the influence of close and direct contact of the material with the bacteria on microbial viability in a standardized in-vitro setting. It is independent of the diffusivity and solubility of the cement, which is an advantage that can be used to overcome certain limitations associated with the agar diffusion method.[9]

The in-vitro evaluation of antimicrobial activity should be customized to simulate different clinical situations. This study aimed to assess the effect of the fresh mix on the bacteria, simulating the clinical situation. However, a delayed antibacterial effect could not be achieved with the agar diffusion method after seven days due to the probable contamination of the plates. For different intervals, antibacterial assessment using an agar diffusion test, previously mixed cement discs should be performed and stored to be tested after different intervals, which do not simulate the clinical situation.[34,35]

However, significant methodological heterogeneity and inadequacy of standardization have been presented, involving different time intervals, handling, and testing parameters with diverse bacterial strains in the literature. [22] Accordingly, in this study, both evaluation methods (agar diffusion and direct contact) were employed to overcome the limitations of each test and verify the antibacterial performance of the calcium fluoride-modified Biodentine against different bacterial strains.

ICP-MS is considered one of the most powerful tools for chemical analysis. Advances in instrumentation and methodology have allowed ICP-MS to accurately and sensitively detect fluoride ions in different biological samples. Compared to most analytical tools, ICP-MS allows quantitative assessment of small amounts of liquid samples without extensive sample preparation procedures.[28]

In the direct contact test, the different experimental Biodentine groups showed decreased bacterial growth for the three tested bacterial strains compared to the control bacteria groups, indicating the effectiveness of Biodentine in combating bacterial growth. Specifically, the 10% modified group showed a significantly higher antibacterial effect than the other biodentine groups against S. mutans and L. acidophilus strains, as illustrated in Table 1 and Figure 1.

On the other hand, the agar diffusion method revealed that the incorporation of 10% CaF_2 into Biodentine caused bacterial inhibition zones against S. mutans and L. acidophilus bacterial strains, comparable to the positive control (antibiotic). Both the unmodified Biodentine and 5% CaF_2 groups showed bacterial inhibition zones, significantly lower than the antibiotic control disc. None of the experimental groups showed antibacterial inhibition zones against the E. faecalis strain compared to the antibiotic-positive control group, as presented in Table 2.

The antibacterial effect observed may be attributed to the alkaline pH resulting from the release of calcium hydroxide as a hydration reaction product in all groups.[22] Adding 10% CaF_2 to Biodentine could improve its antibacterial effect against S. mutans and L. acidophilus. The improvement in the antibacterial performance with the 10% CaF_2 may be referred to as the higher fluoride release with its antibacterial effect, which is concentration-dependent.[12] The higher amount of fluoride released from the 10% groups, along with its alkalizing potential, likely contributes to the enhanced antibacterial potential of this group.[22,36]

A recent systematic review [17] revealed that most studies give evidence that E. faecalis is a major pathogen responsible for endodontic failures. E. faecalis exhibits defense mechanisms to withstand different challenging environments, including disinfectant resistance, biofilm formation, living in unreachable areas in dentinal radicular dentine structure, and synergistic interaction with other strains.[17] The antimicrobial testing results strongly depend on the concentration and contact time within the test, especially the agar diffusion, which may show more resistance of E. faecalis to antimicrobial agents.[37]

Our direct contact test results against E. faecalis are consistent with the findings of Elsaka et al.[21], which revealed that the titanium tetrafluoride-modified Biodentine had an inhibitory effect after 1 hour of mixing and was confirmed for seven days.

The antibacterial results of the present study against E. faecalis agree with the results of Pelpenko et al.[38], which showed that no inhibition halos were observed for the different tricalcium silicate-based cements in the agar diffusion test. Biodentine showed a more significant inhibition zone compared to the other materials. Direct contact with E. faecalis had a significantly higher optical density, indicating increased bacterial growth for all tested cements.

The results of the current study against S. mutans and L. acidophilus using an agar diffusion test align with previous research conducted by Deveci et al.[39], which revealed a high antibacterial efficacy of Biodentine against these bacterial strains.

Our findings partially agree with those of Esteki et al.[26], which assessed the antimicrobial activity of different tricalcium silicate-based cements against E. faecalis, S. mutans, and C. albicans. Esteki et al.[26] revealed that Biodentine has intense antimicrobial activity against the Streptococcus mutans strain. However, it contradicted the present study regarding the antibacterial efficacy of Biodentine against E. faecalis using an agar diffusion test. This contradiction could be attributed to the discrepancies in the agar diffusion test results, which significantly depend on testing condition variability in different test set-ups.

Our results agree with a previous study [40], which found no bacterial inhibition zones around Biodentine against E. faecalis in the agar diffusion test. However, the direct contact method detected antibacterial activity against the same bacteria, confirming the influence of the in vitro testing method on antibacterial results for the same bacterial strain and cement.

It is important to note that the heterogeneity among the results in the literature may be attributed to the different methodological setting parameters such as nutrients, incubation conditions, time, oxygen saturation, and assessment techniques.[34] One of this study's limitations is that it uses of a single-species bacteria model, whereas oral biofilm is more complex clinically. While many direct contact test studies have found several dental materials effective against planktonic cultured bacteria, they could not show their effectiveness against biofilm microorganisms and infected dentine.[40,41]

Fluoride-releasing dental materials are assumed to be effective in caries control, this assumption is still contraversial.[10] The combined antibacterial and fluoride release qualities with its remineralization potential added to Biodentine may improve its clinical performance, especially in the recent conservative techniques that recommend incomplete caries removal, leading to increased bacterial load and remaining infected dentine. [3,20]

Given that microorganisms are considered direct causative factors for the development of carious and pulpal diseases, as well as the failure of endodontic treatment,

the provision of an effective reduction of microorganisms was the target for obtaining a successful antibacterial cement material. The current study revealed that Biodentine can be considered an effective and encouraging antibacterial cement, positively affecting the outcome of restorative and endodontic treatments. Adding 10% CaF_2 to Biodentine appears to be a promising antibacterial and anti-cariogenic cement suitable for different restorative applications. Further studies on the long-term antibacterial effect with different calcium fluoride concentrations, forms of incorporation, and testing protocols are recommended.

Conclusions

Within the limitations of this study, it could be concluded that:

1. Adding 10 % CaF_2 to Biodentine could improve its antibacterial performance against S. mutans and L. acidophilus.
2. Calcium fluoride could not improve the antibacterial performance of Biodentine against E. faecalis.
3. Calcium fluoride could add anti-cariogenic potential with fluoride-releasing qualities to Biodentine.

Ethical approval

This research was done after ethical approval (FDASU-Rec ER122338) from the university's ethical committee.

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Competing interests

The authors declare no competing interests.

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