Journal of Dental Sciences and Education

DOI: 10.51271/JDSE-0083

Original Article

Evaluation of antibacterial activity of different pulp capping materials

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Cite this article as: Sağmak S, Çellik Ö, Akgün S. Evaluation of antibacterial activity of different pulp capping materials. *J Dent Sci Educ.* 2026;4(3):91-96. doi:10.51271/JDSE-0083

Received: 01.07.2026 ◆ **Accepted**: 01.08.2026 ◆ **Published**: 20.08.2026

ABSTRACT

Aims: Pulp health is at risk from environmental factors such as caries or trauma. To prevent these risks, the pulp is treated with a capping material, either directly or indirectly. The aim of this study was to evaluate the antibacterial effects of four different pulp capping materials against *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)*.

Methods: The antimicrobial activity of four different pulp capping materials (TheraCal LC, Dycal, MTA Angelus, and MTA Cem LC) and a chlorhexidine control group was assessed using the agar diffusion method at three-time intervals (1, 3, and 5 days). For each experimental group, twenty samples were prepared and tested against *S. mutans* (ATCC® 25175) and *L. acidophilus* (ATCC® 11975), which served as standard bacterial strains. The obtained data were analyzed using repeated-measures ANOVA followed by Bonferroni post hoc tests for multiple comparisons.

Results: Based on the overall mean values across all measurement periods, the chlorhexidine control group exhibited the highest antimicrobial activity against both *S. mutans* and *L. acidophilus*. Analyzing the experimental groups, MTA Angelus, TheraCal LC, and Dycal showed antimicrobial activity against *S. mutans*. MTA Cem LC demonstrated no discernible antimicrobial activity. However, TheraCal LC and Dycal demonstrated antimicrobial activity against *L. acidophilus*. MTA Angelus and MTA Cem LC exhibited no antimicrobial activity. Statistically significant differences were observed among the groups on all measurement days ($p<0.05$).

Conclusion: The antibacterial performance of pulp capping materials was influenced by both material composition and bacterial species. These findings highlight the importance of considering antibacterial properties alongside biological characteristics when selecting pulp capping materials.

Keywords: Pulp capping, antibacterial activity, *Streptococcus mutans*, *Lactobacillus acidophilus*, mineral trioxide aggregate

INTRODUCTION

Vital pulp therapy aims to preserve the vitality of pulp tissues compromised by deep carious lesions, restorative procedures, or traumatic injuries in the absence of pulp necrosis. This therapeutic approach, commonly referred to as pulp capping, involves the placement of a biocompatible protective material over the exposed pulp to promote tertiary dentin formation.[1]

Accurate assessment of pulp status prior to the capping procedure is critical for determining treatment prognosis. Clinical diagnosis is guided by responses to thermal stimuli, pain on percussion, and radiographic findings.[2] In vital pulp therapy, adequate pulp perfusion and periodontal health are fundamental to preserving pulp vitality. Effective hemorrhage control, thorough disinfection of the cavity walls, and the selection of an appropriate capping material are equally essential for achieving favorable treatment outcomes.[3]

Pulp capping is indicated in cases of deep carious lesions or when pulp exposure results from carious progression. Dental caries arise from the destruction of hard tooth tissues by acidogenic bacteria; therefore, reducing bacterial load and activity is pivotal in arresting carious progression and protecting the pulp. *Streptococcus mutans (S. mutans)* and *Lactobacillus acidophilus (L. acidophilus)* are the principal microorganisms implicated in caries development.[4] *S. mutans*, which plays a central role in caries initiation, is recognized as the predominant cariogenic species in the oral flora. Its presence across various caries types is attributed to its acidogenic and aciduric characteristics.[5] *L. acidophilus* is a gram-positive bacterium that metabolizes sugars to produce lactic acid. Although frequently identified in active carious lesions, it is not considered a primary initiator of caries due to its relatively late colonization; nonetheless, it is implicated in the progression of dentinal caries in advanced stages.[6]

Vital pulp therapy pursues two principal objectives: arresting carious progression and preventing its reactivation. This begins with the elimination of communication between the carious cavity and the oral environment. Following rubber dam isolation, infected carious tissue is removed and bacterial

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contamination is reduced. The subsequent restoration should incorporate antibacterial materials capable of inhibiting bacterial proliferation and metabolic activity.[7]

Materials intended for pulp capping must fulfill a range of essential criteria, including the capacity to stimulate dentin repair, exert antibacterial effects, promote pulp healing, demonstrate biocompatibility, and restrict lesion advancement.[8] In recent years, the development of novel vital pulp treatment materials has generated considerable research interest regarding their antimicrobial properties.

Among the capping materials evaluated in the present study, Dycal is a calcium hydroxide-based cavity liner with well-established clinical use. Its principal properties include acid neutralization, antibacterial activity, stimulation of secondary dentin formation, radiopacity, and ease of clinical application.[9,10] TheraCal LC is a resin-modified, light-cured, calcium silicate-containing pulp capping material suitable for both direct and indirect pulp capping. It provides a protective barrier for the pulp and is composed of calcium oxide, strontium, silica, barium sulfate, calcium silicate, barium zirconate, Bis-GMA, and polydimethacrylate. Its composition consists of approximately 45% mineral content, 10% radiopaque filler, 5% hydrophilic thickener, and 40-45% resin. TheraCal LC is notable for its sustained release of calcium ions.[11] MTA Angelus is a mineral trioxide aggregate-based material whose principal constituents include tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicon oxide. Its indications encompass root canal furcation repair, management of internal resorption, pulpotomy, and both direct and indirect pulp capping.[12] MTA Cem LC is a light-polymerized, resin-modified, tricalcium silicate-based material indicated for use as a protective liner beneath light-cured base materials and as a co-lining agent in deep cavities or cases involving pulp exposure.[13]

Antibacterial activity is assessed through various methodologies, including disk and well agar diffusion tests and dilution assays. Among these, the agar diffusion technique is widely regarded as the method of choice owing to its cost-effectiveness, technical simplicity, and capacity for the simultaneous comparison of multiple test materials.[14]

Although the antibacterial properties of several pulp capping materials have been investigated, limited information is available regarding newly introduced resin-modified calcium silicate-based materials. In particular, the antibacterial activity of MTA Cem LC has not been previously reported. Therefore, the present study aimed to evaluate the antibacterial efficacy of four pulp capping materials-TheraCal LC, MTA Angelus, Dycal, and MTA Cem LC-against *S. mutans* and *L. acidophilus*, two microorganisms actively involved in carious progression and pulp inflammation. The null hypothesis of the study was as follows: there is no statistically significant difference in antibacterial activity against *S. mutans* and *L. acidophilus* among the tested pulp capping materials.

METHODS

Ethics

Since the study was conducted exclusively under laboratory conditions using previously available bacterial strains, Ethics Committee approval was not required in accordance with the applicable institutional and national regulations. All procedures were carried out in accordance with the ethical rules and the principles.

The present study is an in vitro microbiological laboratory study. The bacterial strains used in this research were obtained from the laboratory collection of the Public Health Institution of Turkiye. No human participants, patient samples, clinical specimens, personal data, or animal subjects were involved in the study.

Study Design

This study has been completed at the Faculty of Dentistry, Adıyaman University. The laboratory phase of the study was completed over a three-month period. The aim of the study was to evaluate the antibacterial activity of four experimental groups and one control group against *S. mutans* (ATCC® 25175) and *L.* acidophilus (ATCC® 11975) on days 1, 3, and 5.

Preparation of Samples

An a priori power analysis was conducted using G*Power 3.1.9.7 for a repeated-measures ANOVA (within-between interaction). Based on a large effect size [f(V)=0.50], α=0.05, and 95% power, the minimum required sample size was 50 specimens. Accordingly, 50 specimens were analyzed for *S. mutans* and 50 for *L. acidophilus* (n=10 per group), resulting in a total of 100 specimens. For each experimental group, twenty specimens measuring 4 mm in depth and 7 mm in diameter were fabricated using cylindrical plastic molds (**Table 1**). Specimens in groups 1 and 4 were polymerized using a blue light-emitting LED for the durations specified by the manufacturers. In contrast, specimens in groups 2 and 3 were prepared by manual mixing on a glass slab with a metal spatula and subsequently placed into the molds. All samples were covered with a mylar strip and glass coverslips and allowed to set at 25°C. All procedures during sample preparation were performed under sterile conditions using sterile instruments, and the specimens were aseptically placed onto the appropriate culture media without risk of contamination.

Table 1. Experimental groups, manufacturers and material contents

Material	Manufacturer	Components	LOT number
TheraCal LC	Bisco, Lançon De Provence, France	**Light-curing single paste:** Resin (Bis-GMA and PEGD) modified calcium silicate, filled with CaO, calcium silicate particles (type III Portland cement), Sr glass, fumed silica, barium sulfate, and barium zirconate	2300110466
Dycal	Dentsply Tulsa Dental, Johnson City, TN, USA	Two-paste system made of a base paste (1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, calcium tungstate, and iron oxide pigments) and a catalyst paste (calcium hydroxide, N-ethyl-o/p-toluene sulphonamide, zinc oxide, titanium oxide, zinc stearate, and iron oxide pigments)	023304
MTA Angelus	Angelus, Londrina, Brazil	**Powder:** tri-calcium silicate, di-calcium silicate, tri-calcium aluminate, ferroaluminate, tri-calcium, calcium oxide, bismuth oxide **Liquid:** distilled water	102273
MTA Cem LC	GC, Tokyo, Japan	BisGMA, CaO, silica, barium sulphate ($BaSO_4$)	LC210714

LC: Light-cured, Bis-GMA: Bisphenol A-glycidyl methacrylate, PEGD: Polyethylene glycol dimethacrylate, CaO: Calcium oxide, Sr: Strontium, $BaSO_4$: Barium sulfate

Materials tested:

- **Group 1:** TheraCal LC
- **Group 2:** Dycal
- **Group 3:** MTA Angelus
- **Group 4:** MTA Cem LC
- **Group 5 (Control):** Chlorhexidine (2%, BEST, Turkiye)

Assessment of Antibacterial Activity

For each experimental group, a total of 20 samples were prepared, including 10 samples (n=10) inoculated with *S. mutans* (ATCC® 25175) and 10 samples (n=10) inoculated with *L. acidophilus* (ATCC® 11975) standard bacterial isolates.[11] For the agar diffusion test, sheep blood agar (SBA) (ThermoScientific Oxford, UK) and MRS (Man, Ragosa & Sharpe) media were used. Lyophilized *S. mutans* (ATCC® 25175) and *L. acidophilus* (ATCC® 11975) strains were cultured in appropriate growth media and incubated at 37°C under CO_2-enriched conditions. Following incubation, bacterial suspensions were prepared in physiological saline and adjusted to a 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml). A 100 µL aliquot of the standardized suspension was uniformly spread onto the surface of agar plates using sterile cotton swabs. Wells measuring 7 mm in diameter were created in the agar plates using the blunt end of a micropipette tip, and the wells were completely filled with the test materials (**Figure 1, 2**).

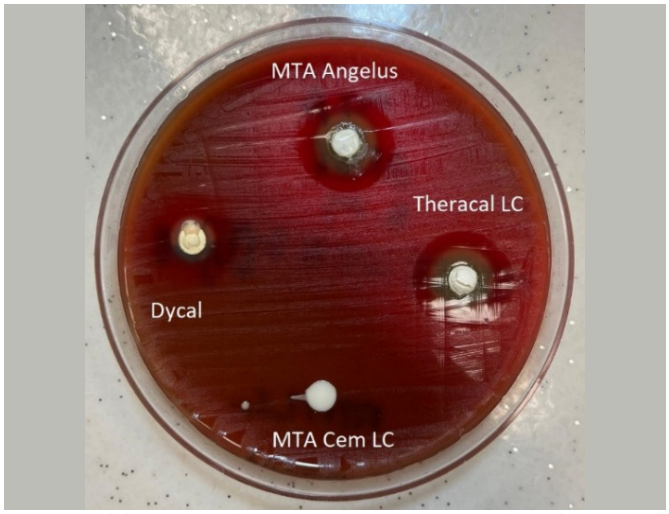

Figure 1. Inhibition zone diameters against *S. mutans* on the 1st day

S. mutans: Streptococcus mutans, MTA: Mineral trioxide aggregate, LC: Light-cured, CEM: Calcium-enriched mixture

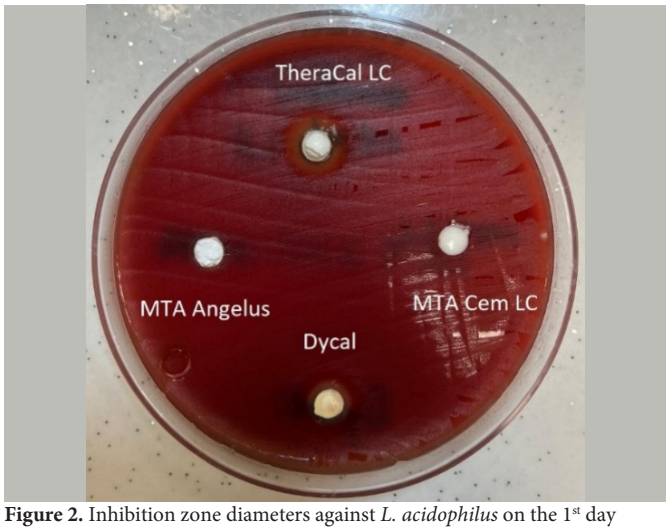

Figure 2. Inhibition zone diameters against *L. acidophilus* on the 1st day

L. acidophilus: Lactobacillus acidophilus, LC: Light-cured, MTA: Mineral trioxide aggregate, CEM: Calcium-enriched mixture

Statistical Analysis

All data analysis of the data obtained in this study was performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. Results were expressed as mean±standard deviation (SD). Changes over time were evaluated using repeated measures analysis of variance (repeated measures ANOVA). Bonferroni post hoc tests were used for multiple comparisons. $p<0.05$ was considered significant.

RESULTS

The antibacterial activity of the tested pulp capping materials against *S. mutans* is presented in **Figure 3**. When the mean values of the three measurement periods were evaluated, the chlorhexidine-treated control group demonstrated the highest antimicrobial activity against *S. mutans*. Among the experimental materials, MTA Angelus showed the greatest antibacterial effect, followed by TheraCal LC and Dycal, respectively. A reduction in antimicrobial effectiveness was observed in subsequent measurements for these groups. In contrast, MTA Cem LC did not exhibit any detectable antimicrobial activity. Statistically significant differences among the groups were identified on all measurement days ($p<0.05$) (**Table 2**).

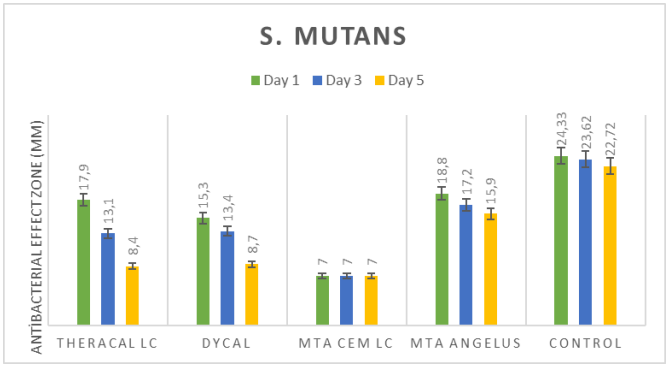

Figure 3. Graphical representation of the effectiveness obtained from the experimental groups against *S. mutans*

S. mutans: Streptococcus mutans, MTA: Mineral trioxide aggregate, CEM: Calcium-enriched mixture, LC: Light-cured

Table 2. Antimicrobial activity values against *S. mutans*

Groups	Day 1	Day 3	Day 5	Mean/SD
TheraCal LC	17.90±0.73^c	13.10±0.99^c	8.40±0.69^c	13.13±1.33^c
Dycal	15.30±1.15^c	13.40±1.34^c	8.70±0.94^c	12.47±0.74^c
MTA Angelus	18.80±1.03^b	17.20±0.63^b	15.90±0.73^b	17.30±0.74^b
MTA Cem LC	7.00±0.00^d	7.00±0.00^d	7.00±0.00^d	7.00±0.00^d
Chlorhexidine	24.33±0.37^a	23.62±0.42^a	22.72±0.66^a	23.60±0.43^a

Values are presented as mean±SD. Different superscript letters indicate statistically significant differences between groups according to Bonferroni post hoc comparisons ($p<0.05$). *S. mutans: Streptococcus mutans*, SD: Standard deviation, LC: Light-cured, MTA: Mineral trioxide aggregate, CEM: Calcium-enriched mixture

The data obtained from the experimental groups against *L. acidophilus* are presented in **Figure 4**. Based on the mean values obtained from three measurement periods, the chlorhexidine-treated control group exhibited the highest antimicrobial activity against *L. acidophilus*. Among the experimental materials, TheraCal LC and Dycal demonstrated antibacterial effects following the control group; however, a reduction in their efficacy was observed in subsequent measurements. In contrast, neither MTA Angelus nor MTA Cem LC showed any detectable antimicrobial activity against

L. acidophilus. Statistically significant differences among all groups were identified at each measurement interval ($p<0.05$) (**Table 3**).

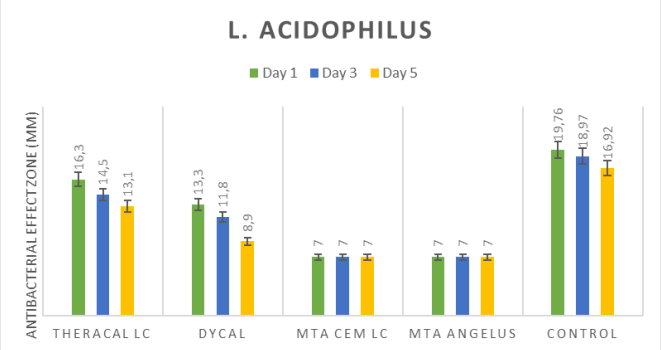

Figure 4. Graphical representation of the data obtained from the experimental groups against *L. acidophilus*

L. acidophilus: Lactobacillus acidophilus, MTA: Mineral trioxide aggregate, CEM: Calcium-enriched mixture, LC: Light-cured

Table 3. Antimicrobial activity values against *L. acidophilus*

Groups	Day 1	Day 3	Day 5	Mean/SD
TheraCal LC	16.30±0.67^{b}	14.50±0.53^{b}	13.10±0.57^{b}	14.63±0.43^{b}
Dycal	13.30±0.95^{c}	11.80±0.92^{c}	8.90±1.20^{c}	11.33±0.90^{c}
MTA Angelus	7.00±0.00^{d}	7.00±0.00^{d}	7.00±0.00^{d}	7.00±0.00^{d}
MTA Cem LC	7.00±0.00^{d}	7.00±0.00^{d}	7.00±0.00^{d}	7.00±0.00^{d}
Chlorhexidine	19.76±0.85^{a}	18.87±0.55^{a}	16.92±1.40^{a}	18.86±0.79^{a}

Values are presented as mean±SD. Different superscript letters indicate statistically significant differences between groups according to Bonferroni post hoc comparisons ($p<0.05$). *L. acidophilus: Lactobacillus acidophilus*, SD: Standard deviation, LC: Light-cured, MTA: Mineral trioxide aggregate

DISCUSSION

Microorganisms play a central role in the etiology of dental caries. The dominant bacteria and their metabolic byproducts in deep dentinal caries that have reached the pulp pose a direct threat to pulp health. Preserving pulp vitality therefore requires the use of a biocompatible material with proven antibacterial properties.[15] Accordingly, *S. mutans* the predominant species in enamel caries and *L. acidophilus* the dominant species in dentinal caries were selected as the test microorganisms in this study.

The agar disk diffusion method is widely employed in dental research owing to its technical simplicity and cost-effectiveness; however, a recognized limitation of this approach is its inability to characterize the specific mechanisms or temporal dynamics of the antimicrobial effect.[14]

Pulp exposure may arise as a complication of prosthetic treatment, trauma, or carious progression. The primary objective of vital pulp therapy is to preserve the health of the pulp tissue. Both direct and indirect pulp capping protect pulp vitality by establishing a biological barrier against thermal, chemical, and microbial insults.[16] The essential function of a capping material is to shield the exposed pulp and sustain its vitality through the stimulation of reparative dentin formation. Calcium hydroxide, a cornerstone of vital pulp therapy, induces a superficial necrotic zone on the exposed pulp surface, thereby triggering hard tissue deposition through repair mechanisms. Simultaneously, calcium hydroxide applied over a thin residual dentin layer undergoes dissolution, facilitating the release of growth factors from the pulp.[17] In addition to organic solvent- or water-based suspensions and calcium salicylate-based paste formulations such as Dycal, resin monomer-based calcium hydroxide preparations are also available. Beyond these conventional materials, contemporary bioactive pulp capping agents have been developed. Bioactive materials are defined as therapeutic agents designed to initiate biomineralization while inherently possessing antibacterial properties.[18] Tricalcium silicate-based materials such as MTA and Biodentine do not contain calcium oxide in their composition; however, calcium hydroxide is generated upon contact with tissue fluids.[19] In the present study, four widely used pulp capping materials were evaluated; TheraCal LC, Dycal, MTA Angelus, and MTA Cem LC.

TheraCal LC is a light-cured, resin-modified, calcium silicate-based pulp capping material. The release of hydroxyl ions (OH^{-}) from TheraCal LC establishes a highly alkaline environment with bactericidal properties.[20] Poggio et al.[21] assessed the antibacterial effects of six pulp capping materials-Dycal, Calcicur, Calcimol LC, TheraCal LC, MTA Angelus, and Biodentine-against three streptococcal species and reported that TheraCal LC demonstrated antibacterial activity against *S. mutans*. Similarly, Fathy et al.[22] investigated the antimicrobial effects of Biodentine and TheraCal LC against *S. mutans* and found that, although Biodentine exhibited greater antibacterial efficacy, the difference between the two materials was not statistically significant. Our findings are consistent with previous reports demonstrating the antibacterial activity of TheraCal LC against *S. mutans*.

Gandolfi et al.[23] reported that TheraCal LC releases higher levels of calcium than ProRoot MTA and Dycal, yet displays considerably lower solubility, while the pH of TheraCal LC was comparable to that of ProRoot MTA. Overall, TheraCal LC has been shown to exhibit antibacterial activity comparable to that of calcium hydroxide and calcium silicate-based materials.[21]

Dycal is a calcium hydroxide-based material used for both direct and indirect pulp capping that sets via a chemical reaction. Upon dissolution in water, it dissociates into calcium and hydroxyl ions. The resulting alkaline environment disrupts bacterial cell wall integrity and induces protein denaturation, thereby conferring antibacterial properties.[10] Moradi et al.,[24] in a comparative study involving Fuji II LC glass ionomer, Dycal, Calcimol LC, TheraCal LC, and ACTIVA BioACTIVE, reported that Dycal exhibited the highest antimicrobial activity among all tested groups.

The elevated pH of MTA results from the sustained release of calcium ions and the subsequent formation of calcium hydroxide. This high alkalinity, combined with significant ion release, underlies the antibacterial properties attributed to MTA.[25] While MTA has demonstrated efficacy against certain facultative bacteria, it has been shown to be ineffective against anaerobic species. Kim et al.[26] found that MTA Angelus exhibited greater antibacterial activity against *S. mutans* compared to two other mineral trioxide aggregate formulations, Endocem MTA and ProRoot MTA.

Elshamy et al.[25] compared the antimicrobial properties of MTA, Dycal, and bioceramic materials against *S. mutans* and *L. acidophilus* using an agar diffusion test, and determined that MTA exhibited the highest level of antimicrobial activity. The authors attributed this finding to the strong correlation

between MTA's elevated pH and its pronounced antibacterial properties.

MTA Cem LC is a recently introduced light-cured, resin-modified, tricalcium silicate-based material indicated for direct and indirect pulp capping as well as for use as a protective base or liner beneath composite restorations, amalgams, cements, and other restorative base materials. To date, no studies evaluating the antibacterial efficacy of this material have been reported in the literature. In the present study, MTA Cem LC did not demonstrate any inhibitory effect on the tested bacteria. This may be attributed to the reduction in ion release associated with its resin content, which likely impairs the degree of pH elevation and consequently diminishes its antimicrobial potential. The absence of published data on this material underscores the need for further investigation. Considering its resin-modified formulation, future studies should evaluate not only its antibacterial activity but also calcium ion release, alkalinity, and bioactivity using complementary in vitro and in vivo models.

Bakir et al.[27] reported that MTA demonstrated antibacterial activity against *S. mutans, L. acidophilus and Enterococcus faecalis (E. faecalis).* Zarrabi et al.[28] similarly reported antibacterial activity of MTA against *E. faecalis* and *S. mutans*.

Poggio et al.[21] conducted a comparative analysis of the antibacterial activity of six pulp dressing materials against *Streptococcus salivarius*, *S. sanguis*, and *S. mutans*. Among the materials tested, MTA Angelus, Calcicur, Dycal, and TheraCal LC demonstrated inhibitory activity against *S. mutans*, with MTA Angelus producing the most pronounced effect, followed by TheraCal LC, Dycal, and Calcicur, in descending order. Biodentine and Calcimol LC did not exhibit any measurable antimicrobial activity.[29] The findings of the present study are in agreement with these results; MTA Angelus was the most effective material against *S. mutans*, followed by TheraCal LC and Dycal, respectively. MTA Cem LC, however, did not demonstrate any antimicrobial effect.

The antibacterial activity of pulp capping materials may be considered a relevant factor in the clinical decision-making process during vital pulp therapy. Although the success of vital pulp therapy is primarily associated with the preservation of pulp vitality and the induction of reparative dentin formation, the presence of residual microorganisms following caries removal remains a potential challenge. Therefore, materials exhibiting antibacterial properties may provide a biological advantage by limiting bacterial activity at the pulp-dentin interface and supporting a more favorable environment for pulpal healing.[15] However, the clinical performance of pulp capping materials is determined by a combination of factors, and antibacterial activity should be evaluated together with other important characteristics, such as biocompatibility, bioactivity, sealing ability, and mechanical properties.[30]

Limitations

This study has several limitations. First, the antibacterial activity of the tested materials was evaluated only under in vitro conditions using the agar diffusion method, which may not fully reflect the complex biological environment of the oral cavity. Moreover, this method primarily evaluates the inhibitory effect of materials and may be influenced by their diffusion properties within the agar medium. Second, only two cariogenic bacterial species were investigated, whereas clinical dental caries is associated with a more diverse microbial community. In addition, the present study focused on antibacterial activity and did not evaluate other biological properties of the materials, such as biocompatibility, bioactivity, or long-term interaction with pulp tissues. Therefore, further in vivo and long-term clinical studies involving broader microbial models and comprehensive biological evaluations are needed to confirm the clinical relevance of the present findings.

CONCLUSION

Within the limitations of this in vitro study, the antibacterial activity of pulp capping materials was found to vary according to both the material composition and the bacterial species tested. While MTA Angelus, TheraCal LC, and Dycal exhibited antibacterial activity against *S. mutans*, only TheraCal LC and Dycal demonstrated activity against *L. acidophilus*. In contrast, MTA Cem LC showed no detectable antibacterial effect against either bacterial species under the experimental conditions. These findings suggest that the antimicrobial performance of pulp capping materials may be influenced by their composition and should be considered alongside their biological and physicochemical properties when selecting a material for vital pulp therapy. Further well-designed in vivo and long-term clinical studies are required to confirm the clinical relevance of these findings.

ETHICAL DECLARATIONS

Ethics Committee Approval

Since the study was conducted exclusively under laboratory conditions using previously available bacterial strains, Ethics Committee approval was not required in accordance with the applicable institutional and national regulations.

Informed Consent

Since the study was conducted without the participation of any living being, no written consent form was obtained.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

Concept: SS, ÖC; Design: SS, ÖC; Control: SS, ÖC; Resources: SS, SA; Materials: SS, SA; Data Collection and/or Processing: SS, SA; Analysis and/or Interpretation: SS, SA; Literature Review: SS, ÖC, SA; Writing the Article: SS; Critical Review: SS, ÖC, SA.

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