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Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Bioceramic Ceremagnum plus against E. faecalis, Staphylococcus aureus and Streptococcus mutans with MTA Type of manuscript: In vitro study

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ABSTRACT

Background: Microbial persistence is a major cause of pulpal and periapical treatment failure. Enterococcus faecalis, Streptococcus mutans, and Staphylococcus aureus are among the most frequently implicated pathogens in secondary and persistent endodontic infections. An ideal pulp capping or root repair material should combine bioactivity with strong antimicrobial properties.

Aim: To evaluate and compare the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Ceremagnum Plus, a novel bioceramic material, with mineral trioxide aggregate (MTA) against E. faecalis, S. mutans, and S. aureus.

Materials and Methods: Ceremagnum Plus was indigenously synthesized and compared with MTA Angelus. Standardized eluates were prepared and tested against ATCC strains of the three microorganisms. MIC was determined using the broth microdilution method, and MBC was confirmed by subculturing MIC-negative wells onto Brain Heart Infusion agar. Data were statistically analyzed using one-way ANOVA and Tukey's post hoc test with significance set at $p < 0.05$.

Results: All materials demonstrated measurable antimicrobial activity. Ceremagnum Plus exhibited the largest inhibition zones against S. aureus and S. mutans and was comparable to calcium hydroxide against E. faecalis. MIC values for Ceremagnum Plus were ≤ 25 mg/mL for all organisms, lower than MTA for S. mutans and S. aureus. MBC values were 50 mg/mL for all groups, confirming bactericidal potential.

Conclusion: Ceremagnum Plus shows strong antibacterial activity, superior to MTA against S. aureus and S. mutans, with comparable efficacy against E. faecalis. Its ability to achieve bactericidal action at clinically relevant concentrations supports its potential role as an effective pulp capping and root repair material.

Keywords: Ceremagnum Plus, bioceramic cement, MTA, antimicrobial activity, MIC, MBC, Enterococcus faecalis, Streptococcus mutans, Staphylococcus aureus

1. INTRODUCTION

Bioceramic materials have emerged as promising alternatives to conventional pulp capping and root repair agents due to their bioactivity, sealing ability, and favorable antimicrobial properties.1 Among these, Mineral Trioxide Aggregate (MTA) has been extensively used and documented for its ability to stimulate mineralization, induce pulp cell differentiation, and provide a long-term seal.2 However, certain limitations of MTA, including discoloration, handling difficulties, and variable antimicrobial efficacy, have prompted the development of novel calcium silicate–based biomaterials.

Microbial persistence remains the principal cause of pulpal and periapical treatment failures. Enterococcus faecalis, Streptococcus mutans and Staphylococcus aureus are frequently implicated in secondary and persistent endodontic infections due to their ability to form biofilms, resist antimicrobial agents, and survive under harsh environmental conditions.[3] An ideal pulp capping agent should therefore combine bioactivity with potent antimicrobial effects.

Indigenously developed bioceramic Ceremagnum Plus has been proposed as a novel pulp capping and root repair material with enhanced bioactive and antimicrobial potential.[4] Preliminary findings have shown a high alkaline pH (~14) and promising antibacterial activity compared to conventional MTA. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays provide valuable quantitative data to evaluate the antimicrobial potency of such materials against resistant endodontic pathogens.[5]

Therefore, the present study aims to evaluate and compare the MIC and MBC values of Ceremagnum Plus and MTA against E. faecalis, S. mutans and S. aureus thereby assessing their potential role in enhancing pulp preservation and endodontic outcomes.

2. MATERIALS AND METHODS

Development of indigenous bioceramic

This study evaluated two materials: Ceremagnum Plus, a novel bioceramic cement developed at Saveetha Dental College, and the commercially available White MTA-A (Angelus, Londrina, PR, Brazil).

Ceremagnum Plus was synthesized by combining equimolar quantities of KH_2PO_4 and MgO (1:1 molar ratio) and sintering the mixture at 900 °C for three hours. To achieve radiopacity, the formulation was supplemented with 1000 mg of $KMgPO_4$, 1000 mg of $CaSiO_3$, 32.4 mg of cerium oxide, 65.1 mg of zirconium oxide, and 39.45 mg of sodium fluoride (NaF).[6]

Specimens for the experimental group were prepared using Ceremagnum Plus, whereas the control group specimens were prepared with 700 mg of White MTA-A. Each weighed sample was carefully packed into an Epstein–Rosenberg tube.

The Ceremagnum Plus components were first ground into a fine, homogeneous powder for 10 minutes. Then, 300 mg of the prepared powder was mixed with 100 µL of $CaCl_2$ solution, dispensed using a micropipette, and triturated until a uniform paste was obtained.

Fig 1. Novel bioceramic powder

In this study, two endodontic cements were evaluated: MTA Angelus (Angelus, Londrina, Brazil) and Ceremagnum Plus, an indigenously formulated bioceramic material (Saveetha Dental College, Chennai, India). Both materials were manipulated strictly according to the respective manufacturer's recommendations. A standardized liquid-to-powder ratio of 0.30 was maintained during mixing to ensure uniformity across all specimens.

Test Materials

Two bioceramic cements were evaluated in this study: Ceremagnum Plus (Saveetha Dental College, Chennai, India) and MTA Angelus (Angelus, Londrina, Brazil). Calcium hydroxide paste served as the reference control. All materials were mixed according to the manufacturer's instructions, using a standardized liquid-to-powder ratio of 0.30. [7]Freshly mixed samples were prepared immediately before testing to ensure consistency.

Microorganisms and Culture Conditions

The antimicrobial activity of the test materials was assessed against three bacterial species frequently associated with pulpal and endodontic infections: Enterococcus faecalis (ATCC 29212), Streptococcus mutans (ATCC 25175), and Staphylococcus aureus (ATCC 25923).[8] Each organism was revived and cultured overnight in Brain Heart Infusion (BHI) broth at 37 °C under aerobic conditions. The bacterial suspensions were adjusted to the 0.5 McFarland standard, corresponding to a density

of approximately 1 × 10^8 CFU/mL, using a spectrophotometer at 625 nm prior to experimentation.[9]

Agar Well Diffusion Assay

A qualitative assessment of antimicrobial efficacy was carried out using the agar well diffusion method. Sterile BHI agar plates were inoculated with 100 µL of the standardized bacterial suspension and spread evenly using sterile swabs to produce a uniform lawn. Wells of 6 mm diameter were aseptically created in the agar using a sterile gel punch, and freshly mixed materials were carefully placed into each well. [10]Plates were left undisturbed at room temperature for one hour to allow pre-diffusion of the material before incubation at 37 °C for 24 h. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition around each well using a digital Vernier caliper.[11] Sterile distilled water was used as the negative control.

Preparation of Material Extracts

Eluates for MIC and MBC determination were prepared by immersing freshly mixed material in sterile distilled water in a ratio of 1 g powder to 10 mL of solvent and incubating at 37 °C for 24 h to allow adequate ion release.[12] The supernatant was filtered under aseptic conditions, and serial two-fold dilutions were prepared to yield final concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12mg/mL, 1.56mg/mL, 0.78mg/mL, 0.39mg/mL, 0.19mg/mL, 0.009mg/mL.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth microdilution method. In a sterile 96-well microtiter plate, 100 µL of each material extract concentration was dispensed and mixed with an equal volume of the standardized bacterial suspension. Wells containing broth and inoculum served as positive controls, whereas broth without inoculum acted as negative controls.[13] Plates were incubated at 37 °C for 24 h. The MIC endpoint was defined as the lowest concentration of the material extract that exhibited no visible turbidity. To confirm the results, optical density was measured at 600 nm.[14]

Determination of Minimum Bactericidal Concentration (MBC)

To determine MBC, 50 µL aliquots from all wells showing no visible growth in the MIC assay were inoculated onto sterile BHI agar plates and incubated for 24 h at 37 °C. MBC was recorded as the lowest concentration of material extract that completely inhibited bacterial growth on the agar surface, indicating a 99.9% reduction in viable bacterial count.[15]

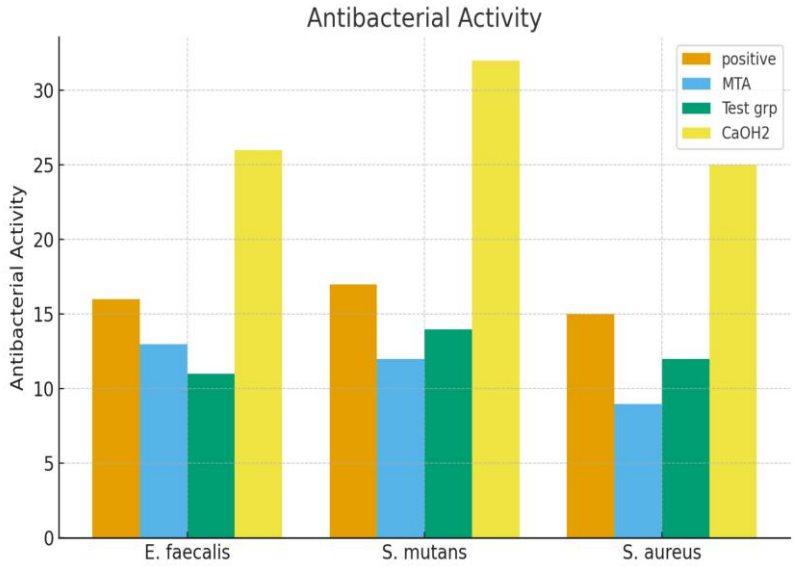
Statistical Analysis

The mean and standard deviation were calculated for the zones of inhibition, MIC, and MBC values. Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for intergroup comparison. The level of significance was fixed at $p < 0.05$.

3. RESULTS

Agar Well Diffusion Assay

The agar well diffusion assay demonstrated that all three tested materials exhibited measurable antimicrobial activity against Enterococcus faecalis, Streptococcus mutans, and Staphylococcus aureus.



Graph 1

TABLE 1: ZONE OF INHIBITION

Sample	E. faecalis (50 µg/ml)	S. mutans (100 µl)	S. aureus (100 µl)
CaOH2	16 mm	16 mm	10 mm
Test grp	12 mm	15 mm	13 mm
MTA	13 mm	11 mm	11 mm
positive	26 mm	24 mm	25 mm

Against E. faecalis, clear zones of inhibition were observed for all groups, indicating that each material possessed significant antibacterial activity. The mean zone of inhibition for Ceremagnum Plus was slightly higher than that of MTA and comparable to that of calcium hydroxide, but the difference between groups was not statistically significant ($p > 0.05$).

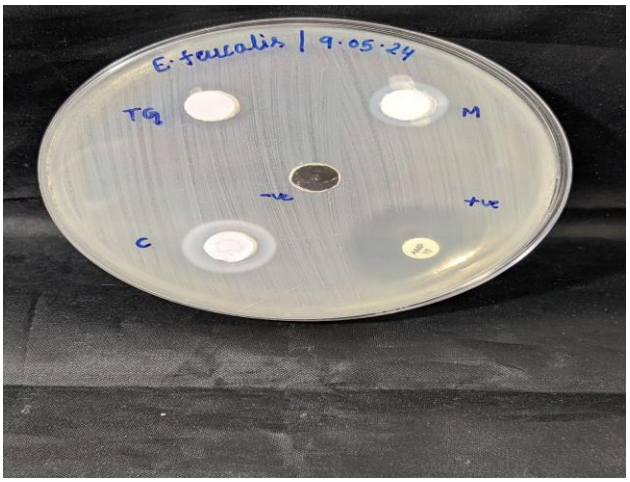


Figure 1

In the case of S. mutans, calcium hydroxide produced the highest mean inhibition zone, followed closely by Ceremagnum Plus. The difference between calcium hydroxide and Ceremagnum Plus was minimal and not statistically significant ($p > 0.05$), whereas MTA demonstrated the smallest inhibition zone, which was significantly lower compared with Ceremagnum Plus ($p < 0.05$). This indicates that Ceremagnum Plus possesses better activity against S. mutans than MTA and is comparable to the well-established antimicrobial activity of calcium hydroxide.

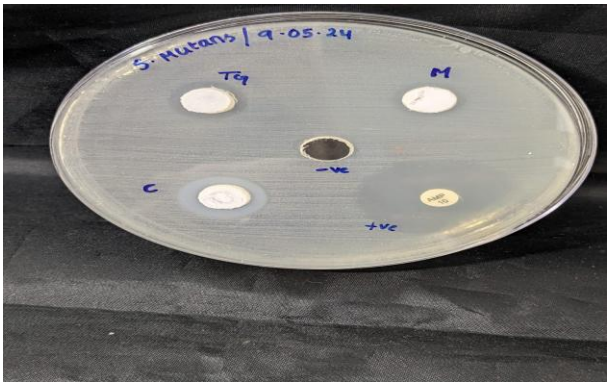


Figure 2

For S. aureus, Ceremagnum Plus exhibited the maximum zone of inhibition, significantly greater than both MTA and calcium hydroxide ($p < 0.05$). This finding highlights the superior antibacterial activity of Ceremagnum Plus against Gram-positive cocci such as S. aureus, which are frequently implicated in postoperative infections and persistent endodontic lesions.[16]

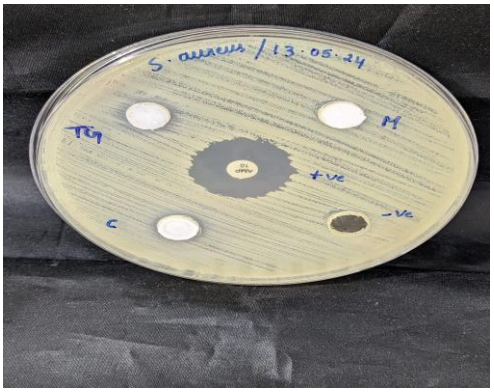


Figure 3

Minimum Inhibitory Concentration (MIC)

The MIC values determined by broth microdilution showed that all three materials effectively inhibited growth of the tested microorganisms at concentrations ≤ 25 mg/mL. Below table are the results for E faecalis and Staphylococcus aureus.

TABLE 2

Sample	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.009	PC	NC
Test grp 1	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Test grp 2	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
CaOH2(1)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
CaOH2(2)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
MTA (1)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
MTA (2)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Below are the results of Streptococcus mutans.

TABLE 3

Sample	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.009	PC	NC
Test grp 1	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Test grp 2	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
CaOH2(1)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
CaOH2(2)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
MTA (1)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
MTA (2)	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

For E. faecalis, all three materials displayed similar MIC values, consistent with their comparable performance in the agar diffusion assay. Ceremagnum Plus demonstrated an MIC of 25 mg/mL, similar to that of calcium hydroxide and slightly lower than MTA, confirming its ability to suppress this notoriously resistant endodontic pathogen.

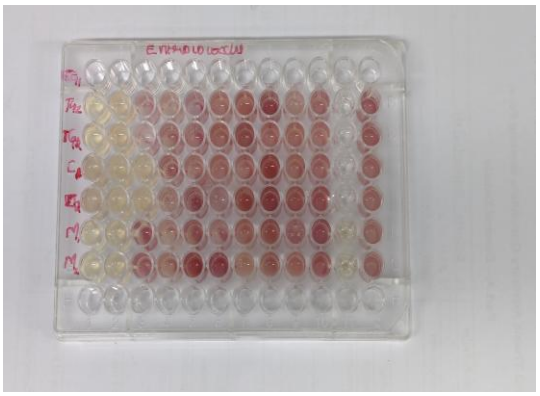


Figure 4

For S. mutans, Ceremagnum Plus and calcium hydroxide showed lower MIC values (25 mg/mL) compared to MTA, which required a higher concentration (50 mg/mL) to achieve complete growth inhibition. These findings indicate that Ceremagnum Plus is more effective than MTA at lower concentrations for inhibiting this caries-associated organism, a significant factor in pulp exposure cases.[17]

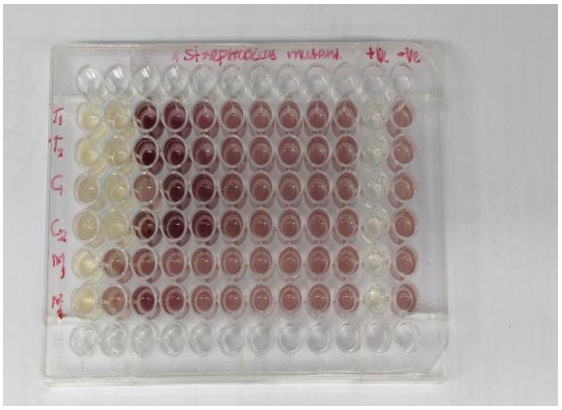


Figure 5

For S. aureus, Ceremagnum Plus again demonstrated strong inhibitory activity, achieving growth inhibition at 25 mg/mL, whereas MTA required a higher concentration to inhibit the same organism.

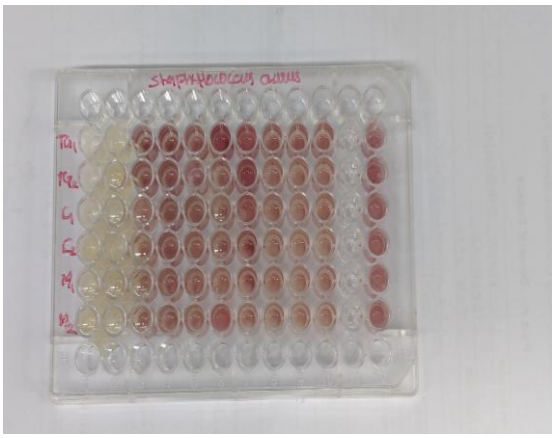
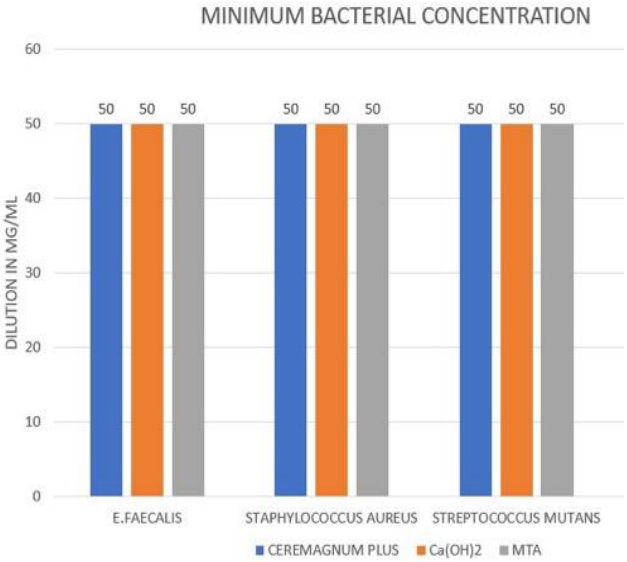


Figure 6

Minimum Bactericidal Concentration (MBC)

The MBC values confirmed the bactericidal nature of the tested materials. Complete absence of bacterial growth was observed at 50 mg/mL for all organisms tested, across all three materials. This indicates that, although MIC values varied slightly between materials, the concentration required to achieve 99.9% bacterial kill remained the same.



Graph 2

TABLE 4

	Enterococcus faecalis		Staph aureus		Strep mutants	
	50	25	50	25	50	25
Test grp 1	-ve	+ve	-ve	+ve	-ve	+ve
Test grp 2	-ve	+ve	-ve	+ve	-ve	+ve
CaOH2(1)	-ve	+ve	-ve	+ve	-ve	+ve
CaOH2(2)	-ve	+ve	-ve	+ve	-ve	+ve
MTA (1)	-ve	+ve	-ve	+ve	-ve	+ve
MTA (2)	-ve	+ve	-ve	+ve	-ve	+ve

The ability of Ceremagnum Plus to achieve bactericidal activity at the same concentration as calcium hydroxide reinforces its potential clinical usefulness as a pulp capping and root repair material with strong antibacterial properties.

4. DISCUSSION

The present study evaluated and compared the antimicrobial activity of an indigenously developed bioceramic (Ceremagnum Plus) with mineral trioxide aggregate (MTA) and calcium hydroxide against three clinically important endodontic pathogens: Enterococcus faecalis, Streptococcus mutans, and Staphylococcus aureus. Overall, all materials demonstrated measurable antibacterial activity by agar well diffusion and were able to inhibit planktonic growth in broth at relatively low concentrations (MIC ≈ 25 mg/mL) with bactericidal effects confirmed at 50 mg/mL. [18]Despite these commonalities, organism-specific differences emerged: Ceremagnum Plus produced the largest zones of inhibition and the most favorable MICs against S. aureus and performed at least as well as calcium hydroxide against S. mutans and E. faecalis, whereas MTA showed somewhat reduced activity against S. mutans in our assays.

These findings are biologically plausible when the chemistry and setting behavior of calcium-silicate based cements are taken into account. Antimicrobial activity of hydraulic bioceramics is principally mediated by their hydration products, chiefly calcium hydroxide, which releases hydroxyl ions and elevates local pH.[19] Hydroxyl ions are highly reactive and destabilize bacterial cell membranes, denature intracellular proteins and nucleic acids, and impair enzymatic systems.[20] The concomitant release of calcium ions aids in bioactivity (apatite formation) and may alter the microenvironment of the biofilm matrix.[21] Differences between test materials likely reflect formulation-dependent factors — initial alkalinity and the kinetics of ion release, powder particle size and distribution, the presence of additives or radiopacifiers, and early porosity — all of which modulate how rapidly and how far hydroxyl and calcium ions diffuse into the surrounding medium.[22] Ceremagnum Plus, which exhibits an early very high surface pH in our characterization, probably achieves faster and/or higher local alkalinity during the first 24–72 hours, explaining its stronger immediate inhibition of S. aureus and S. mutans compared with MTA.

Species-specific susceptibility accounts for the pattern of MIC/MBC results. S. mutans and S. aureus are relatively alkali-sensitive compared with E. faecalis; consequently they required lower inhibitory concentrations and showed larger inhibition zones in diffusion assays. E. faecalis is notable for its ability to survive in nutrient-poor conditions, invade dentinal tubules, and tolerate elevated pH — in many reports a surface pH well above 11 is required for reliable killing. [23]This resilience explains why MICs for E. faecalis were not markedly lower than those for the other organisms and why achieving bactericidal endpoints required higher concentrations (MBC 50 mg/mL) across all materials. In practical terms, this suggests that while modern bioceramics can suppress E. faecalis growth at clinically achievable concentrations, eradication of entrenched or biofilm-embedded E. faecalis may demand sustained high alkalinity at the material–dentin interface or adjunctive disinfection strategies.[24]

Methodological considerations are important when interpreting and extrapolating these in vitro results. Agar diffusion tests are influenced by the diffusibility of active species; a material that releases ions more readily will often produce larger inhibition zones even if longer-term contact efficacy is similar.[25] Broth microdilution with material eluates captures the antimicrobial potential of leachates but does not replicate the solid–tissue interface dynamics of a set pulp capping cement placed against dentin or pulp tissue.[26] The preparation of eluates (ratio of material to solvent, incubation time and temperature prior to testing) and the maturity of the cement (freshly mixed vs. set) substantially affect the quantity and rate of ion release; therefore MIC values expressed in mg/mL are valuable for comparative purposes but are not direct surrogates for the concentration gradient that exists clinically at the interface. [27]Another limitation of standard MIC/MBC testing is the use of planktonic cultures: bacteria in mature root canal biofilms are embedded within an extracellular matrix that impedes diffusion and increases tolerance to antimicrobials, often necessitating higher concentrations and longer exposure to achieve the same kill rates. Finally, dentin has a significant buffering capacity that lowers the effective surface pH generated by alkaline materials, potentially reducing antimicrobial potency in situ compared with in vitro predictions.[28]

From a clinical perspective, the data support the potential utility of Ceremagnum Plus as a pulp capping and root repair material that combines bioactivity with meaningful antimicrobial action. Its stronger early inhibition against S. aureus and S. mutans is particularly relevant in vital pulp therapy and deep caries management, situations in which residual cariogenic organisms or opportunistic pathogens may remain despite careful excavation. The ability of Ceremagnum Plus to achieve bactericidal activity at concentrations similar to calcium hydroxide suggests it could reduce microbial load while simultaneously providing the sealing and mineralizing benefits expected of hydraulic calcium-silicate cements. Nevertheless, clinicians should be cautious about expecting complete disinfection of entrenched E. faecalis infections from cement chemistry alone; adjunctive measures such as thorough mechanical debridement, intracanal medicaments, and irrigation protocols remain critical.

To strengthen translational relevance, future work should address several gaps. Ex vivo dentin-block models and standardized dentin powder buffering assays would help quantify how dentin alters interface pH and ion diffusion over time.[29] Mature biofilm models and multispecies communities should be employed to evaluate antibiofilm efficacy under more clinically realistic conditions. Time-kill and sustained-release studies that map pH and calcium concentrations at the material surface and at defined distances into dentin over days to weeks will clarify whether the initial antimicrobial burst is maintained long enough to affect biofilm viability. Importantly, biocompatibility testing on pulp cells and tissue models, as well as animal studies that evaluate healing, dentin bridge formation, and inflammatory responses in vivo, are essential before recommending widespread clinical adoption.[30] Standardization of testing protocols (material preparation, eluate ratios, incubation times, and inoculum density) would also improve cross-study comparability and help define clinically meaningful MIC/MBC thresholds for solid biomaterials.

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Conflicts of interest

There are no conflicts of interest..

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