Original Article

Comparative evaluation of the antibacterial efficacy of two experimental calcium silicate-based intracanal medicaments: An *in-vitro* study

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Abstract

Introduction: Success of endodontic treatment relies on minimizing microbial load by chemo-mechanical preparation and intra-canal medication(ICM). Calcium hydroxide based ICMs have known disadvantages. Calcium silicate-based cements(CSC) exhibit antibacterial activity, thus promoting researchers to experiment with their formulations to use them as ICMs.

Aim: Evaluation and comparison of the antimicrobial efficacy of two experimental CSC (MTA & Biodentine + 2%chlorhexidine) and Bio-C Temp against E.faecalis.

Methods and Material: Test materials were divided into four groups namely Group1-Bio-C Temp, Group2-UltraCAL XS, Group3-Biodentine+2%CHX and Group4-MTA+2%CHX. Direct contact test was done by placing a standardized suspension of E.faecalis on test materials and bacterial growth was assessed spectrophotometrically using ELISA at one, three and seven days.

Statistical Analysis: Data was analysed using one-way ANOVA, Tukey's multiple post hoc test and paired-t test. Results: Intragroup comparison revealed decreased mean optical density(OD) in groups 1, 2, and 4; no significant difference in group 3. Intergroup comparison showed statistical differences in mean OD values between groups (3 and 4); groups (1 and 2) at days one(p-0.018) and three(p-0.035), but no difference individually. Group 4 showed the highest antimicrobial efficacy on day seven.

Conclusion: MTA+2%CHX & Biodentine+2%CHX showed better antimicrobial efficacy and hence could be used as potential ICMs.

Keywords: Antimicrobial efficacy; Bio-C Temp; biodentine +2% chlorhexidine; calcium silicate-based cements; *Enterococcus faecalis*; mineral trioxide aggregate +2% chlorhexidine; spectrophotometry; UltraCAL XS

INTRODUCTION

Achieving effective minimization of microbial load within the root canal complex is asserted as a pivotal element for the success of endodontic treatment.[1] Merely shaping the canal is insufficient for thorough bacteria elimination, prompting the recommendation of a combined approach involving irrigation, instrumentation, and intracanal medication.[2] Studies have shown that a significant proportion, ranging from 40% to 60%, of cultivable bacteria remains present in the root canals, even after undergoing irrigation with sodium hypochlorite.[3]

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Enterococcus faecalis has been identified as the primary bacterial agent in cases of apical periodontitis. This Gram-positive microorganism, acknowledged for its resistance, tends to persist in secondary root canal infections.[4] Notably, *E. faecalis* possesses a unique trait of independently developing a single-species biofilm within the root canal, without the need for interaction with other microbial species. It has demonstrated heightened tenacity to chemo-mechanical disinfection and additional therapies administered during root canal treatment.[3] In conjunction with root canal disinfection, intracanal medicaments are crucial in enhancing the disinfection protocol.[5] Achieving optimal disinfection of the root canal complex is essential to prevent tissue destruction caused by the release of bacteria or their by-products into the periapical region.[3] In endodontics, a range of intracanal medicaments, such as antibiotics, calcium hydroxide (CH), iodoform-containing pastes, steroids, and chlorhexidine (CHX), are employed for this purpose.[2] CH has conventionally been favored as an intracanal therapeutic agent for its antibacterial properties and ability to stimulate mineralized tissue deposition, attributed to hydroxyl and calcium ions.[6] UltraCal XS (Ultradent Products, USA), a CH-based intracanal medication, exhibits antibacterial properties, promotes mineralization, deactivates bacterial lipopolysaccharides, and is bio-inert.[5] Despite the advantages, CH-based medicaments are susceptible to tissue fluids, soluble in root canals, and have potential adverse effects on tooth fracture resistance after prolonged use.[5] In addition, their efficacy against specific facultative bacteria, such as *Candida albicans* and *E. faecalis*, commonly found in secondary endodontic pathology, may be limited. The intricate spatial distribution of CH around the canal poses challenges for complete clearance.[2]

Calcium silicate-based cement (CSC) has grown in popularity and is now the preferred material for a variety of endodontic operations, including root defect repair (perforations and resorptions), root-end bridging material, root canal sealant, and pulp capping agent. In addition, CSC exhibits antibacterial activity through the discharge of hydroxide ions from calcium silicate, leading to an increase in pH values. The potential utilization of CSC as an intracanal medicament thus warrants further exploration.[2]

Bio-C Temp (Angelus, Brazil) is a bioceramic material that uses calcium silicate and tungstate as radiopacifiers. According to the manufacturer's literature, it is safe to use as an intracanal medicament. A study discovered that, while Bio-C Temp displayed comparable cytocompatibility at higher dilutions and more or comparable activation of alkaline phosphatase activity and mineralized nodule formation when compared to UltraCal XS, it had much less antibacterial and antibiofilm action.[5]

CSCs, including mineral trioxide aggregate (MTA) and biodentin, have broad applications in dentistry, but their potential as intracanal medicaments is limited by their setting reaction. To enhance antibacterial effectiveness, researchers have explored substituting the mixing liquid in CSC with a microbiocidal agent. Combining 2% CHX with MTA powder notably improved the antibacterial efficacy of white and gray MTA formulations against *E. faecalis*.[7,8] Modifying the setting of CSCs by replacing the mixing liquid, such as with a mixture of MTA and 2% CHX, delays the setting reaction, releasing more calcium ions and exhibiting improved flow compared to CH.[2] Experiments by Kogan *et al*.[9] demonstrated a 4 h delay in setting with the MTA and CHX mixture, while Mahmoud *et al*.[2] found an 84-day delay with 2% CHX in CSCs.

Given the limited antibacterial effectiveness observed in the commercially available CSC (Bio-C Temp), as an intracanal medicament, there is a demand for an improved CSC formulation. Currently, there are lacunae in the literature regarding a comparative analysis of the antibacterial efficacy between Bio-C Temp and experimental CSC intracanal medicaments. Therefore, this study aimed to assess the antibacterial activity of two experimental CSC formulations and juxtapose them with Bio-C Temp. The null hypothesis proposed that there would be no discernible differences among the intracanal medicaments under investigation.

MATERIALS AND METHODS

The study comprised four primary groups outlined as follows:

- Group 1: Bio-C Temp (Angelus, Londrina, PR, Brazil)
- Group 2: UltraCal XS (Ultradent Products Inc., South Jordan, UT, USA)
- Group 3: Biodentine™ (Septodont) mixed with 2% CHX (Consepsis™ V) in a 1:1 ratio
- Group 4: MTA (ProRoot MTA, Dentsply) mixed with 2% CHX (Consepsis™ V) in a 1:1 ratio.

Test microorganism

E. faecalis (ATCC 29212) was cultivated on a BHI agar plate and then subcultured on a nutritional agar medium. Following confirmation of the strain's purity, a bacterial suspension was produced in 5 ml of saline and spectrophotometrically adjusted at 800 nm to reach a transmittance equal to 90T (which equates to 1.5×10^8 CFUs on the 0.5 McFarland scale) [Figure 1a].

Direct contact test

In this investigation, we employed the direct contact test, which focuses on evaluating bacterial growth turbidity within 96-well microtiter plates. In our present study, we generated new combinations of all test substances and placed them at the bottom of four wells in the microtiter plate (subgroup A) with a height of 2 mm. Subsequently, a 10 µl bacterial suspension was applied to these

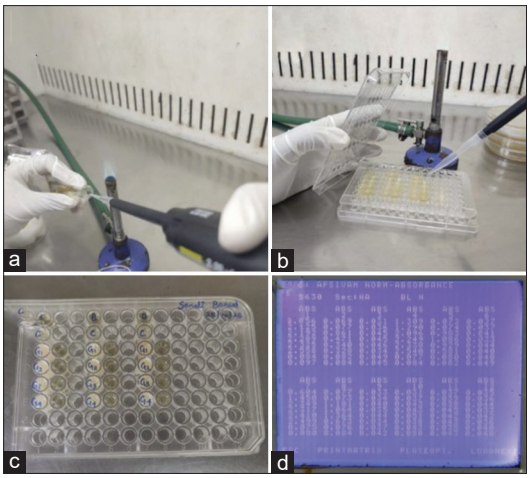

Figure 1: (a) *Enterococcus faecalis* culture, (b) Transfer of brain heart infusion broth to the microtiter wells, (c) Direct contact test being carried out in 96 well microtiter plates, (d) Optical density reading

combinations. To facilitate direct interaction between bacteria and the test material, 245 µl of BHI broth was added [Figure 1b]. Following a 2-min mixing interval, 15 µl of the resulting mixture was transferred into four adjoining wells (subgroup B), each containing 215 µl of fresh medium [Figure 1c]. The temporal dynamics of bacterial growth in individual wells were continuously monitored using an ELISA reader set at 630 nm. Densitometric readings were documented on the 1st, 3rd, and 7th days for each specific set of samples [Figure 1d]. To ensure the reliability of the results, the experiments were repeated three times.[10]

RESULTS

Data analysis was carried out using the SPSS (Statistical Package for the Social Sciences) version 21. The normality of the data was examined using the Shapiro–Wilk test. Inference statistics included the implementation of a one-way analysis of variance, followed by the *post hoc* Tukey's test. The predetermined level for statistical significance was set at 0.05.

Table 1 depicts the intergroup comparison of optical density values among the test groups on days 1, 3, and 7. On days 1 and 3, there was an increase in the OD values in Groups 1 and 2 and a decrease in the OD values in Group 3 and Group 4, the difference of which was a statistical difference (0.018, 0.035). Individually between (Group 1 and Group 2) and (Group 3 and Group 4), there was no statistical difference (0.121, 0.827) on days 1 and 3. On day 7, there was no statistical difference (0.280) among any of the groups. The results inferred that in Groups 1 and 2, on days 1 and 3, there was decreased antibacterial activity, which subsequently increased by day 7. There was significant antibacterial activity for Groups 3 and 4 from day 1, which persisted till day 7. Among the groups, Group 4 showed the highest antimicrobial efficacy at all time intervals.

Table 2 depicts the intragroup comparison of optical density values among the test groups on days 1, 3, and 7. Comparison showed that the mean OD values decreased from day 1 to day 3 and subsequently from day 3 to day 7 in all the groups, but these differences failed to reach the level of statistical significance ($P < 0.01$).

DISCUSSION

The primary goal of endodontic treatment is the elimination of bacterial biofilm within the root canal system, a key factor in pulpal and periapical diseases. Inadequate removal of these microorganisms during procedures can result in infections, a major cause of root canal treatment failures. The intricate dentin composition and anatomical complexities of the root canal system present challenges for achieving complete disinfection, particularly in persistent infections. Relying solely on chemical disinfection may be insufficient, underscoring the critical importance of selecting an appropriate intracanal medicament to comprehensively eradicate microorganisms in such cases.[3]

The prevalence of *E. faecalis* in persistent root canal infections, as determined by polymerase chain reaction, ranges from 67% to 77%. Consequently, *E. faecalis* was chosen as the test organism in this study.[11]

The research employed the direct contact test methodology outlined by Weiss *et al.*,[12] which emphasizes direct and close proximity between the test entity and the material under examination, irrespective of the solubility and diffusibility of antimicrobial components. This method facilitates testing water-insoluble materials under various conditions, including aging. Notably, the direct contact test is a reliable and reproducible qualitative approach that effectively controls confounding factors and is unaffected by the size of the inoculum interacting with the test material.[13]

In the current investigation, CH has been employed as the control test material. It is chosen as the gold standard and widely recognized as the most commonly used intracanal medication.[14]

The primary objective of this study was to evaluate the antibacterial efficacy of two experimental CSCs and to compare them with the commercially available CSC (Bio-C Temp). The obtained results led to rejecting the null hypothesis, indicating variations in the antibacterial efficacy among the intracanal medicaments tested.

Table 1: Intergroup comparison of optical density values on day 1, day 3, and day 7

	*n**	Mean	SD	95% CI for mean		*P*	*Post hoc* pairwise comparison
				Lower bound	Upper bound		
Day 1							
Group 1	3	1.3633	0.04726	1.2459	1.4807	0.018, significant†	Group 1, Group 2>Group 3, Group 4
Group 2	3	1.3200	0.03464	1.2339	1.4061		
Group 3	3	0.7267	0.02517	0.6642	0.7892		
Group 4	3	0.6000	0.14731	0.2341	0.9659		
Day 3							
Group 1	3	0.9867	0.19858	0.4934	1.4800	0.035, significant†	Group 1, Group 2>Group 3, Group 4
Group 2	3	1.0767	0.07234	0.8970	1.2564		
Group 3	3	0.5867	0.06110	0.4349	0.7384		
Group 4	3	0.6200	0.01732	0.5770	0.6630		
Day 7							
Group 1	3	0.7767	0.02887	0.7050	0.8484	0.280, NS‡	-
Group 2	3	0.6600	0.22338	0.1051	1.2149		
Group 3	3	0.6533	0.32517	−0.1544	1.4611		
Group 4	3	0.4000	0.09539	0.1630	0.6370		

*Number of specimens, †Statistically significant with $P<0.05$, ‡Statistically nonsignificant with $P>0.05$. Statistical analysis: One-way ANOVA, Tukey's test. SD: Standard deviation, NS: Nonsignificant, CI: Confidence interval

Table 2: Intragroup comparison of optical density values

	*n**	Group 1		Group 2		Group 3		Group 4	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 1	3	1.3633	0.04726	1.3200	0.03464	0.7267	0.02517	0.6000	0.14731
Day 3	3	0.9867	0.19858	1.0767	0.07234	0.5867	0.06110	0.6200	0.01732
Day 7	3	0.7767	0.02887	0.6600	0.22338	0.6533	0.32517	0.4000	0.09539
P		0.06, NS‡		0.05, NS‡		0.0368, NS‡		0.097, NS‡	

*Number of specimens, †Statistically significant with $P<0.05$, ‡Statistically nonsignificant with $P>0.05$. Statistical analysis: One-way ANOVA, Tukey's test. SD: Standard deviation, NS: Nonsignificant

The present study showed that CHX (2%) incorporation enhanced the antimicrobial properties of MTA and biodentine significantly [Table 1] as compared to Bio-C Temp and UltraCal XS from day 1 itself. By day 7 [Table 2], all groups demonstrated similar antimicrobial activity. In the experimental period, MTA with CHX (2%) performed the best, and Bio-C Temp, the poorest.

The findings of the current study indicate that the incorporation of 2% CHX to CSC exhibits no repressive effect on calcium discharge or pH values, which is consistent with earlier findings. Holt *et al*. discovered that combining 2% CHX with MTA enhances the bactericidal activity of MTA against *E. Faecalis*.[7] Mittag *et al*. concluded that MTA with 2% CHX had a broader radius of inhibition against *E. faecalis* than lower doses.[8] Ramezani *et al*. demonstrated decreased bacterial leakage when MTA was mixed with 0.12% CHX.[15] Furthermore, because pH values are accountable for the material's antibacterial action and biological activity, MTA's superior performance could be attributed to the maintenance of a high pH due to the continual release of calcium and Ca(OH)2 production from MTA.[2]

CHX, a bis-biguanide, has bacteriostatic activity at low doses and bactericidal effects at higher doses. The optimal qualities of CHX as an intracanal medicament are mostly owing to its alkaline pH. The incorporation of 2% CHX gel showed improved antibacterial properties. The CHX's positive charge interacts with the negatively charged phosphate clusters on bacterial cell walls, affecting the cell's osmotic balance. Subsequently, the cell wall permeability gradually rises, allowing the CHX ion to breach into the bacteria and weaken their cell walls, particularly the lipopolysaccharide. According to research, the supplementary effect is mostly caused by the fragmentation process, which releases a variety of metabolites from CHX. Because these byproducts possess a higher pH, they act as both an antioxidant and a prooxidant, which enhances their effects.[2]

When manipulating biodentine, changing the protocol in terms of the kind and quantity of liquid mixed with the powder, affects the material's physical and chemical properties, as well as its surface topography, as opposed to biodentine blended according to the manufacturer's instructions, thus, resulting in lower calcium ion release. In our study, this could be the reason for the inferior performance of biodentine to MTA.[16]

The diminished antimicrobial efficacy observed in Bio-C Temp in this study is consistent with the findings of the research conducted by Guerreiro *et al*., where they observed that Bio-C Temp had significantly lower antibacterial activity than UltraCal XS. Bio-C Temp undergoes a hydration reaction in which the calcium silicate-based bioceramics

form calcium silicate gel and CH. The poor microbiocidal effect of Bio-C Temp could be accredited to the generation of sparse CH molecules during the hydration event.[5]

The findings of this study must be interpreted within the context of its limitation, acknowledging that investigations focusing on single or dual microbial species oversimplify the intricate root canal ecology and may not accurately reflect clinically achievable outcomes. The root canal system hosts a diverse array of microorganisms, and the *in vitro* antibacterial efficacy of test agents may face challenges in the complex oral cavity environment. Therefore, further research involving *in vivo* applications of the same medicament is essential to provide a comprehensive understanding. Additionally, to fully assess the potential of combining CHX with MTA and biodentine, further studies should explore the physical properties of these materials.

CONCLUSION

CHX (2%) incorporation enhanced the antimicrobial activity of MTA and biodentine significantly as compared to Bio-C Temp and UltraCal XS. MTA with CHX (2%) performed better than biodentine.

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Conflicts of interest
There are no conflicts of interest.

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