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Comparative Evaluation of Antimicrobial Properties of Different Pulpotomy Agents: An in Vitro Study

[1]Aarya S Kumar, Post Graduate Student, Department of Pediatric and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bangalore, Karnataka

[2]Anitha Anand, Reader, Department of Pediatric and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bangalore, Karnataka

[3]Sunil Raj N, Fmr HOD, Department of Pediatric and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bangalore, Karnataka

[4]Sapna Konde, HOD, Department of Pediatric and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bangalore, Karnataka

Corresponding Author: Aarya S Kumar, Post Graduate Student, Department of Pediatric and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bangalore, Karnataka

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Abstract

Aim: To evaluate and compare the antibacterial efficacy of four pulpotomy agents—Mineral Trioxide Aggregate (MTA), Biodentine, TheraCal PT, and e-IodoCal—against S. mutans, S. aureus, and E. faecalis using an in vitro agar diffusion method over three time intervals (24, 48, and 72 hours).

Materials and Methods: A total of 150 samples were divided into five groups (four experimental, one control), each tested against the three bacterial species. The agar diffusion test was employed to determine antibacterial activity, with inhibition zones measured at 24, 48, and 72 hours. Inhibition zone diameters were analyzed using one-way ANOVA, with Tukey's and Bonferroni's post hoc tests for pairwise comparisons and error control.

Results: Biodentine exhibited the highest and time-dependent antibacterial activity across all microbes. MTA ranked second with consistent efficacy. e-IodoCal showed moderate effectiveness, particularly improving against S. aureus and E. faecalis over time. TheraCal PT had the lowest activity, though efficacy increased modestly against S. mutans and E. faecalis.

Conclusion: Biodentine is the most effective pulpotomy material in terms of antibacterial activity, followed by MTA, e-IodoCal, and TheraCal PT. These findings support Biodentine as the preferred choice when antimicrobial efficacy is critical.

Keywords: Pulpotomy; Biodentine, MTA, Theracal PT, E-Iodocal, Antibacterial Efficacy, Agar Diffusion Test, Pediatric Endodontics, Streptococcus Mutans, Staphylococcus Aureus, Enterococcus Faecalis.

Introduction

Oral health is integral to overall well-being, with dental caries recognized as the most prevalent non-communicable disease globally, affecting billions, including 560 million children [1]. In pediatric dentistry, maintaining the integrity of the primary dentition is critical for proper jaw growth, speech, and aesthetics [2]. Dental caries, primarily due to poor oral hygiene, can lead to pulp exposure and infections, compromising tooth vitality. Preserving the dental pulp—an innervated and vascularized tissue—is vital, as it plays a central role in tooth nutrition, repair, and sensory function [1].

Pathogenic bacteria such as Streptococcus mutans, Enterococcus faecalis, and Staphylococcus aureus are key contributors to dental caries and endodontic infections. Their acidogenic properties, resistance to antimicrobials, and biofilm formation make them difficult to eradicate, often compromising pulp vitality and endodontic outcomes [3,4]. Vital pulp therapy (VPT), particularly pulpotomy, is a minimally invasive technique used to remove infected coronal pulp while preserving the radicular pulp [5]. The success of pulpotomy depends heavily on the properties of the medicament used, especially its antibacterial effectiveness [6].

Calcium hydroxide (CH) has long been the gold standard in pulp therapy due to its antibacterial and regenerative properties, though its success in primary teeth declines over time [7]. Innovations such as e-IodoCal (a CH-based paste with iodoform and silicone oil), Mineral Trioxide Aggregate (MTA), and Biodentine have addressed many of CH's limitations. MTA promotes hard tissue formation and has excellent sealing and biocompatibility, while Biodentine offers fast setting, high bioactivity, and dentin-like properties [8]. Despite MTA's effectiveness, it has drawbacks like crown discoloration and extended setting time [9].

TheraCal PT, a dual-cure, resin-modified calcium silicate material, was developed to overcome the shortcomings of earlier resin-based pulp capping agents [10]. It shows promising cytocompatibility and bioactivity in vitro but lacks long-term clinical validation. Given the persistent need for an ideal pulpotomy material that balances antimicrobial potency, biocompatibility, and physical strength, comparative studies are essential.

This study aims to evaluate and compare the antimicrobial properties of MTA, Biodentine, TheraCal PT, and e-IodoCal to guide evidence-based material selection in pediatric endodontics.

Materials and Methodology

This study assessed the antimicrobial efficacy of four widely used pulpotomy agents:

- MTA (Angelus): Composed of Portland cement, bismuth oxide, and various calcium silicates, MTA is known for its biocompatibility, hard tissue induction, and antimicrobial potential.
- Biodentine (Septodont): Consisting of tricalcium silicate, calcium carbonate, and zirconium oxide in powder form, and calcium chloride with polycarboxylate in liquid, Biodentine offers bioactivity, excellent sealing, and antibacterial properties.
- TheraCal PT (BISCO): A resin-modified calcium silicate material with BISGMA, barium zirconate, and initiators, TheraCal PT is light-curable, easy to apply, and shows promising antimicrobial effects.
- e-IodoCal (Kids-e Dental): Made of calcium hydroxide, iodoform, and silicone oil, e-IodoCal is

valued for its high alkalinity and long-standing use in pediatric endodontics.

Preparation of Bacterial Cultures

The antimicrobial activity of each pulpotomy agent was tested against Streptococcus mutans, Enterococcus faecalis, and Staphylococcus aureus, which are key pathogens in dental and endodontic infections. Pure bacterial strains were obtained from a microbiology lab and subcultured in nutrient broths to ensure optimal growth. A standardized bacterial concentration of 20 µL per liter was achieved using a spectrophotometer to maintain consistency across all tests.

Evaluation of Antimicrobial Activity Using the Agar Well Diffusion Method

Bacterial suspensions of S. mutans, E. faecalis, and *S. aureus* were spread onto blood agar or Mueller-Hinton agar plates using the spread plate technique to create uniform bacterial lawns. After drying for 10–15 minutes, 6 mm × 4 mm wells were created under aseptic conditions and filled with freshly prepared pulpotomy agents; saline-filled wells served as negative controls. The plates were then left at room temperature for 15–20 minutes to allow diffusion of the materials into the agar. Incubation was carried out at 37°C under anaerobic conditions for S. mutans and E. faecalis, and aerobic conditions for S. aureus. Antimicrobial activity was assessed by measuring the diameter of inhibition zones at 24, 48, and 72 hours using a digital caliper. Photographic documentation was performed to support comparative analysis.

Statistical Analysis

Statistical analysis was conducted using SPSS version 20 (IBM SPASS statistics [IBM corp. released 2011]). Inhibition zone diameters were analyzed using one-way ANOVA, with Tukey's and Bonferroni's post hoc tests for pairwise comparisons and error control. Chi-square tests were used for qualitative variables, and t-tests or Mann-Whitney tests were applied for quantitative comparisons. A 5% significance level was used, with additional tests conducted as needed.

Results

Each sample was tested against all three bacterial species, resulting in a comprehensive assessment (10 samples × 5 groups × 3 bacterial strains). The groups were categorized as

- Group 1: Mineral Trioxide Aggregate (MTA)
- Group 2: Biodentine
- Group 3: TheraCal PT
- Group 4: e-IodoCal
- Group 5: Negative Control

Table 1: Comparison of mean Zone of Inhibition (in mm) for S. Mutans b/w groups at different time intervals using One-way ANOVA Test

Time	Groups	N	Mean	SD	Min	Max	p-value
24 hrs.	Group 1	10	12.41	0.62	11.5	13.5	<0.001*
	Group 2	10	17.05	0.47	16.5	18.0	
	Group 3	10	7.39	0.33	7.0	8.0	
	Group 4	10	10.50	0.40	10.0	11.2	
48 hrs.	Group 1	10	15.21	0.43	14.5	16.0	<0.001*
	Group 2	10	21.15	0.35	20.5	21.5	

	Group 3	10	8.65	0.32	8.0	9.0	
	Group 4	10	13.50	0.40	13.0	14.2	
72 hrs.	Group 1	10	17.91	0.47	17.3	18.5	
	Group 2	10	25.05	0.38	24.5	25.5	<0.001*
	Group 3	10	10.25	0.22	10.0	10.5	
	Group 4	10	15.55	0.43	15.0	16.2	

The mean zone of inhibition for *S.Mutans* with different groups at different time intervals using One-Way ANOVA test is shown in Table 1. The antibacterial activity of the four pulpotomy agents against *S. mutans* showed statistically significant differences at all time intervals (24, 48, and 72 hours), with Group 2 (Biodentine) consistently demonstrating the highest mean zones of inhibition. All groups showed increasing antibacterial effectiveness over time, with Group 1 (MTA) ranking second, followed by Group 4 (e-IodoCal), and Group 3 (TheraCal PT) showing the least activity.

Table 2: Comparison of mean Zone of Inhibition (in mm) for S. Aureus b/w groups at different time intervals using One-way ANOVA Test

Time	Groups	N	Mean	SD	Min	Max	p-value
24 hrs.	Group 1	10	12.00	0.82	11.0	13.0	
	Group 2	10	17.90	0.99	16.0	19.0	<0.001*
	Group 3	10	7.30	0.95	6.0	9.0	
	Group 4	10	10.00	0.82	9.0	11.0	
48 hrs.	Group 1	10	13.60	0.97	12.0	15.0	
	Group 2	10	21.20	1.03	20.0	23.0	<0.001*
	Group 3	10	9.00	0.82	8.0	10.0	
	Group 4	10	12.30	0.95	11.0	14.0	
72 hrs.	Group 1	10	15.60	1.08	14.0	17.0	
	Group 2	10	23.80	1.03	22.0	25.0	<0.001*
	Group 3	10	10.30	1.06	9.0	12.0	
	Group 4	10	13.90	0.99	12.0	15.0	

The mean zone of inhibition for *S.Aureus* with different groups at different time intervals using One-Way ANOVA test is shown in Table 2. Group 2 (Biodentine) consistently demonstrated the strongest antibacterial activity against *S. aureus* across 24, 48, and 72 hours, with significantly higher mean zones of inhibition compared to all other groups ($p < 0.001$). Group 1 (MTA) and Group 4 (e-IodoCal) showed moderate and comparable efficacy, while Group 3 (TheraCal PT) remained the least effective at all time intervals.

Table 3: Comparison of mean Zone of Inhibition (in mm) for E. Faecalis b/w groups at different time intervals using One-way ANOVA Test

Time	Groups	N	Mean	SD	Min	Max	p-value
24 hrs.	Group 1	10	10.80	0.79	10.0	12.0	<0.001*
	Group 2	10	17.90	0.99	16.0	19.0	
	Group 3	10	7.20	0.79	6.0	8.0	
	Group 4	10	9.20	1.03	8.0	11.0	
48 hrs.	Group 1	10	13.10	0.99	12.0	15.0	<0.001*
	Group 2	10	21.00	1.05	19.0	22.0	
	Group 3	10	9.00	0.67	8.0	10.0	
	Group 4	10	10.70	0.95	9.0	12.0	
72 hrs.	Group 1	10	15.40	1.08	14.0	17.0	<0.001*
	Group 2	10	24.20	0.92	23.0	25.0	
	Group 3	10	11.20	0.79	10.0	12.0	
	Group 4	10	13.10	0.88	12.0	14.0	

The mean zone of inhibition for *E. Faecalis* with different groups at different time intervals using One-Way ANOVA test is shown in Table 3. Group 2 (Biodentine) consistently showed the highest antibacterial activity against *E. faecalis* at 24, 48, and 72 hours, with statistically significant differences ($p < 0.001$) compared to the other groups. Group 1 (MTA) displayed moderate efficacy, Group 4 (e-IodoCal) showed slightly lower inhibition, and Group 3 (TheraCal PT) remained the least effective throughout the study.

Table 4: Comparison of mean Zone of Inhibition (in mm) for S. Mutans b/w different time intervals in each group using Repeated Measures of ANOVA Test followed by Bonferroni's post hoc Test

Groups	Time	N	Mean	SD	p-value [a]	Sig. Diff	p-value [b]
Group 1	24 hrs.	10	12.41	0.62	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	15.21	0.43		24 h vs 72 h	<0.001*
	72 hrs.	10	17.91	0.47		48 h vs 72 h	<0.001*
Group 2	24 hrs.	10	17.05	0.47	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	21.15	0.35		24 h vs 72 h	<0.001*
	72 hrs.	10	25.05	0.38		48 h vs 72 h	<0.001*
Group 3	24 hrs.	10	7.39	0.33	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	8.65	0.32		24 h vs 72 h	<0.001*
	72 hrs.	10	10.25	0.22		48 h vs 72 h	<0.001*
Group 4	24 hrs.	10	10.50	0.40	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	13.50	0.40		24 h vs 72 h	<0.001*
	72 hrs.	10	15.55	0.43		48 h vs 72 h	<0.001*

The comparison of mean zone of inhibition for *S.Mutans* between different time intervals in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc is shown in Table 4. All four pulpotomy agents showed a significant increase in antibacterial activity against *S. mutans* over time, with Group 2 (Biodentine) consistently demonstrating the highest mean zones of inhibition at 24, 48, and 72 hours ($p < 0.001$). Group 1 (MTA) ranked second, followed by Group 4 (e-IodoCal) with moderate activity, while Group 3 (TheraCal PT) showed the lowest efficacy despite gradual improvement across time intervals.

Table 5: Comparison of mean Zone of Inhibition (in mm) for S. Aureus b/w different time intervals in each group using Repeated Measures of ANOVA Test followed by Bonferroni's post hoc Test

Groups	Time	N	Mean	SD	p-value [a]	Sig. Diff	p-value [b]
Group 1	24 hrs.	10	12.00	0.82	<0.001*	24 h vs 48 h	0.02*
	48 hrs.	10	13.60	0.97		24 h vs 72 h	<0.001*
	72 hrs.	10	15.60	1.08		48 h vs 72 h	0.005*
Group 2	24 hrs.	10	17.90	0.99	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	21.20	1.03		24 h vs 72 h	<0.001*
	72 hrs.	10	23.80	1.03		48 h vs 72 h	<0.001*
Group 3	24 hrs.	10	7.30	0.95	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	9.00	0.82		24 h vs 72 h	<0.001*
	72 hrs.	10	10.30	1.06		48 h vs 72 h	0.001*
Group 4	24 hrs.	10	10.00	0.82	<0.001*	24 h vs 48 h	<0.001*
	48 hrs.	10	12.30	0.95		24 h vs 72 h	<0.001*
	72 hrs.	10	13.90	0.99		48 h vs 72 h	<0.001*

The comparison of mean zone of inhibition for *S. Aureus* between different time intervals in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc is shown in Table 5. All four pulpotomy agents showed significant, time-dependent increases in antibacterial activity against *S. aureus*, with Group 2 (Biodentine) consistently exhibiting the highest mean zones of inhibition at 24, 48, and 72 hours ($p < 0.001$). Group 1 (MTA) and Group 4 (e-IodoCal) demonstrated moderate efficacy, while Group 3 (TheraCal PT) had the lowest activity but showed steady improvement over time.

Table 6: Comparison of mean Zone of Inhibition (in mm) for E. Faecalis b/w different time intervals in each group using Repeated Measures of ANOVA Test followed by Bonferroni's post hoc Test

Groups	Time	N	Mean	SD	p-value [a]	Sig. Diff	p-value [b]
Group 1	24 hrs.	10	10.80	0.79	<0.001*	24 h vs 48 h	0.02*
	48 hrs.	10	13.10	0.99		24 h vs 72 h	<0.001*
	72 hrs.	10	15.40	1.08		48 h vs 72 h	0.005*
Group 2	24 hrs.	10	17.90	0.99	<0.001*	24 h vs 48 h	<0.001*

	48 hrs.	10	21.00	1.05		24 h vs 72 h	<0.001*
	72 hrs.	10	24.20	0.92		48 h vs 72 h	<0.001*
Group 3	24 hrs.	10	7.20	0.79		24 h vs 48 h	<0.001*
	48 hrs.	10	9.00	0.67	<0.001*	24 h vs 72 h	<0.001*
	72 hrs.	10	11.20	0.79		48 h vs 72 h	0.001*
Group 4	24 hrs.	10	9.20	1.03		24 h vs 48 h	<0.001*
	48 hrs.	10	10.70	0.95	<0.001*	24 h vs 72 h	<0.001*
	72 hrs.	10	13.10	0.88		48 h vs 72 h	<0.001*

The comparison of mean zone of inhibition for *E. Faecalis* between different time intervals in each group using repeated measures of ANOVA test followed by Bonferroni's post hoc is shown in Table 6. All pulpotomy agents showed significant time-dependent increases in antibacterial activity against *E. faecalis*, with Group 2 (Biodentine) consistently exhibiting the highest mean zones of inhibition at 24, 48, and 72 hours ($p < 0.001$). Group 1 (MTA) ranked second, followed by Group 4 (e-IodoCal) with moderate efficacy, while Group 3 (TheraCal PT) showed the lowest overall effectiveness despite steady improvement over time.

Discussion

Pulpotomy is a conservative treatment aimed at preserving the vitality of the radicular pulp, particularly valuable in pediatric dentistry for maintaining primary teeth until natural exfoliation [11,12]. Its success is compromised by microbial invasion, especially by *S.* mutans, E. faecalis, and S. aureus. S. mutans plays a central role in dental caries due to its acidogenic, aciduric, and biofilm-forming capabilities. *E. faecalis*, known for its resistance and ability to survive in harsh conditions and penetrate dentinal tubules, is often linked to persistent endodontic failures. *S. aureus*, while less common, can act as a secondary invader, forming biofilms and releasing tissue-damaging toxins, with MRSA strains presenting additional treatment challenges.

An ideal pulp capping agent should prevent microbial leakage, support healing, and exhibit strong antibacterial activity [13]. Given the limitations of traditional agents and the persistence of residual bacteria after caries removal, materials used for pulp therapy must be both biocompatible and antimicrobial. The agar well diffusion method, though limited in reflecting in vivo behavior [14,15,16], remains a reliable tool for evaluating antimicrobial efficacy. This study used this method to assess Biodentine, MTA, e-IodoCal, and TheraCal PT against the aforementioned bacterial species, aiming to identify the most effective agent for microbial control in vital pulp therapy.

Biodentine emerged as the most effective agent, demonstrating the largest and most consistent zones of inhibition (ZOI) against all tested bacteria. Its high pH and bioactive calcium release contribute to both microbial suppression and tissue regeneration. MTA followed closely, showing time-dependent antimicrobial action, particularly due to its sustained ion release and alkalinity. e-IodoCal displayed moderate efficacy, aided by the dual effects of calcium hydroxide and iodoform, while TheraCal PT showed the least antimicrobial activity, likely limited by its resin content and slower ion release.

All tested materials showed time-dependent increases in antimicrobial activity, with Biodentine proving most effective overall. MTA remains a strong alternative due

to its well-documented performance and biocompatibility. e-IodoCal is a viable option in less severe cases, while TheraCal PT, though easy to use, may require formulation enhancements for better microbial control. The findings support Biodentine as a preferred material for pulpotomy in pediatric dentistry, emphasizing the need for further research to optimize clinical outcomes and improve underperforming materials like TheraCal PT.

Conclusion

This in vitro study concluded that Biodentine exhibited the highest and most consistent antibacterial efficacy among four tested pulpotomy materials, making it the preferred choice for pediatric pulp therapy, followed by MTA, with e-IodoCal and TheraCal PT showing moderate to minimal effectiveness.

References

1. Immich F, de Oliveira D, Ribeiro de Andrade JS, da Silva Barboza A, Cuevas-Suárez CE, da Silva AF, et al. Evaluation of antimicrobial properties, cell viability, and metalloproteinase activity of bioceramic endodontic materials used in vital pulp therapy. J Funct Biomater. 2024 Mar 14;15(3):70.
2. Divya S, Sujatha S. An insight on the success of various pulpotomy medicaments in pediatric dentistry—a review of literature. Res J Pharm Technol. 2018;11(6):2647–55.
3. Bakır EP, Bakır Ş, Ünal S. Comparison of antibacterial effects of pulp capping materials. Selcuk Dent J. 2021;8(2):553–60.
4. de Lima Guimarães NL, Otoch HM, de Andrade LC, Ferreira CM, Rocha MM, de Almeida Gomes F. Microbiological evaluation of infected root canals and their correlation with pain. Rev Sul-Bras Odontol. 2012;9(1):31–7.
5. Laser MN, Alsadi TH, Rodriguez FM, Rodriguez SM. Pulpotomy in primary teeth: Biodentine™ versus calcium hydroxide. A systematic review and meta-analysis. Saudi Dent J. 2024 Aug 11.
6. Pimenta HC, Borges ÁH, Bandeca MC, Neves AT, Fontes RG, da Silva PV, et al. Antimicrobial activity of filling materials used in primary teeth pulpotomy. J Int Oral Health. 2015 Apr;7(4):54.
7. Cruz Hondares T, Hao X, Zhao Y, Lin Y, Napierala D, Jackson JG, et al. Antibacterial, biocompatible, and mineralization-inducing properties of calcium silicate-based cements. Int J Paediatr Dent. 2024 Nov;34(6):843–52.
8. Eissa MS, Ibrahim SH, Abdelfattah MY, Elawady BA. Antimicrobial activity of different bioactive therapeutic pulp capping materials with relevance to their biological and clinical consideration: a comparative in vitro study. Egypt Dent J. 2024 Apr 1;70(2):2107–16.
9. Varghese NS, Jeevanandan G, Rajeshkumar S. Comparative evaluation of antimicrobial properties of a novel calcium silicate cement: an in vitro study. J Coast Life Med. 2022 Aug 22;10:97–103.
10. Kim M, Lee SH, Shin DH. In vitro study of the biological and physical properties of dual-cure resin-modified calcium silicate-based cement. Dent J. 2023 May;11(5):120.
11. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986 Dec;50(4):353–80.
12. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006 Feb;32(2):93–8.

13. Haskell EW, Stanley HR, Chellemi J. Direct pulp capping treatments: long-term results. Oper Dent. 1978;3(3):103–8.
14. Poggio C. In vitro antibacterial activity of different pulp capping materials. J Clin Pediatr Dent. 2025;47(5):489–93.
15. Poggio C. Cytocompatibility and antibacterial properties of capping materials. J Clin Pediatr Dent. 2025;47(5):494–8.
16. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999;32(5):361–9.