SHORT REPORT

# ANTIMICROBIAL EFFICACY OF PULPOTOMY MATERIALS AGAINST CARIOGENIC BACTERIA: AN IN VITRO COMPARATIVE STUDY

Noura A Alessa

Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

**Abstract.** The presence of cariogenic bacteria adversely affects pulp therapy outcome by directly influencing the healing process and long-term prognosis of treated teeth. This study compared the antimicrobial efficacy of five commonly used pulp therapy materials, namely formocresol (FC), NeoMTA®, NeoPUTTY™, ProRoot® MTA, and zinc oxide-eugenol (ZOE), against four cariogenic bacteria, *Actinomyces viscosus, Lactobacillus acidophilus, Lactobacillus rhamnosus,* and *Streptococcus mutans*. All five materials exhibited antibacterial activity against the test bacteria. FC demonstrated the most potent bactericidal property, particularly against *L. acidophilus* and *S. mutans*, followed by ZOE, then the mineral trioxide aggregate (MTA)-based materials. However, other properties, such as bioactivity and biocompatibility, must also be considered before recommending the use of these materials in pulpotomy.

**Keywords:** antibacterial, cariogenic bacteria, primary teeth, pulp therapy, pulpotomy material

Correspondence: Noura Alessa, Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Riyadh 11545, Saudi Arabia
Tel: +966 506414147 E-mail: Nalessa@ksu.edu.sa

## INTRODUCTION

The primary objective of a pediatric dentist is to preserve the integrity and functionality of primary teeth; therefore, infected teeth should remain disease-free during endodontic treatment until normal exfoliation occurs (Pimenta *et al*, 2015). Caries in primary teeth can progress to the dental pulp, exposing it to cariogenic bacteria (Bjørndal *et al*, 2019). Bacteria commonly associated with dental caries are *Actinomyces viscosus, Lactobacillus acidophilus, Lactobacillus rhamnosus,* and *Streptococcus mutans,* which play significant roles in the development and progression of dental decay, contributing to the demineralization of tooth structure and potentially leading to pulp exposure (Naushad *et al*, 2023). These bacteria are present in deep carious lesions and release inflammatory mediators, which compromise the vitality and integrity of human dental pulp, potentially inducing necrosis. Consequently, preventing bacterial infection is essential for improving treatment outcome in cavities with deep caries (Bjorndal *et al*, 2010; Simancas-Pallares *et al*, 2010).

Primary teeth pulp therapy is crucial for preventing tooth loss and undesirable tooth movement following extraction (Coll *et al*, 2017). One specific treatment, pulpotomy, is to maintain primary teeth by controlling inflammation and preserving the health of the radicular pulp (American Academy of Pediatric Dentistry, 2025 – could not be verified; see the REFERENCES section) corrected and link was added. This procedure involves the amputation of the coronal pulp tissue while retaining the vitality and integrity of the radicular pulp, followed by the application of an appropriate dental bactericidal material (Camp and Fuks, 2006). Therefore, enhancing the clinical success of vital or non-vital pulp therapy in primary teeth is essential, and this begins with selecting a material that possesses effective bactericidal properties. The ideal material should be biocompatible,

promote healing, preserve the radicular pulp, be non-damaging to pulpal tissue, not interfere with the physiological process of root resorption, and/or present any clinical or radiographic symptoms (Bossù *et al*, 2020).

Various materials are used as bactericidal agents for pulpotomy, with Buckley's formocresol (FC), introduced in 1904, being the most commonly used material for this procedure due to its ease of use and high clinical success rate (Patchett *et al*, 2006; Chakraborty *et al*, 2018). Another option for pulpotomy treatment, is calcium hydroxide ($Ca(OH)_2$), since it is biocompatible and its alkaline property makes it an antimicrobial agent. Calcium hydroxide stimulates the tertiary dentin formation (Katsamakis *et al*, 2013; Oliveira *et al*, 2013); however, it is associated with a risk of inducing internal dentinal resorption (Oliveira *et al*, 2013). Zinc oxide-eugenol (ZOE) is frequently used in primary teeth pulpotomy as it reduces pain through an analgesic effect and promotes pulpal healing (Gonzalez-Lara *et al*, 2016). More recently, mineral trioxide aggregate (MTA) has gained popularity in pulpotomy due to its biocompatibility, excellent sealing properties, ability to stimulate hard tissue production, and high clinical and radiographic success rates (Güven *et al*, 2013).

Accordingly, we compared the antimicrobial efficacy of commonly used pulp therapy materials against 4cariogenic bacteria, aiming to evaluate their ability to inhibit the growth of microorganisms commonly associated with pulp infections. This comparison is essential for identifying materials that can effectively reduce bacterial contamination, which is a critical factor in the healing process and long-term success of pulp therapy. The findings will help guide clinicians in selecting materials that not only support pulpal healing but also effectively control infection, ultimately enhancing the quality and predictability of pediatric dental treatments.

## MATERIALS AND METHODS

### Materials

Five pulp therapy materials were tested, namely FC (Genuine Dental Supply, Los Alamitos, CA), NeoMTA® (NuSmile, Houston, TX), NeoPUTTY™ (NuSmile, Houston, TX), ProRoot® MTA (Tulsa Dental Specialties, Tulsa, OK), and ZOE (Dentsply DeTrey, Konstanz, Germany).

### Bacteria cultures

The cariogenic bacteria used were *Actinomyces viscosus* (ATCC 15987), *Lactobacillus acidophilus* (ATCC 4356), *L. rhamnosus* (ATCC 53103), and *Streptococcus mutans* (ATCC 25175). *A. viscosus*, *L. acidophilus*, *L. rhamnosus*, and *S. mutans* was cultivated at 37°C for 24 hours. *A. viscosus* was cultivated in Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI), *L. acidophilus* and *L. rhamnosus* in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid Ltd, Basingstoke, UK), and *S. mutans* in BHI broth (Difco Laboratories, Detroit, MI), all at 37 °C for 24 hours under appropriate atmospheric conditions. Antibacterial activity was determined using a disc diffusion method according to the protocols of the Clinical and Laboratory Standards Institute (CLSI, 2023). Three equidistant holes, each with a diameter of 3.5 mm and a depth of 4 mm, were created in each plate, approximately 15 mm from the edges (total of 24 wells in 8 plates). Each tested material was mixed with a sterile spatula on a sterile glass slab according to the manufacturer's instructions. Some materials used in this study as NeoMTA®, ProRoot® MTA, and ZOE were supplied in powder and liquid components and prepared in accordance with the manufacturer's specified mixing ratios. All preparation procedures were done based on their instructions., placed into the holes using a sterile carrier, and gently pressed into position. All plates were maintained at room temperature for 2 hours to allow for the diffusion of the tested materials

and subsequently incubated at 37°C for 48 hours. A lawn of bacteria was grown on brain heart infusion agar (Difco Laboratories, Detroit, MI). The inhibition zone diameter (mm) was measured using a caliper. Each assay was carried out in triplicate, with saline as a negative control.

**Statistical analysis**

Evaluation of statistical significance among the inhibition diameters employed one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test. A $p$-value <0.05 is considered significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 26 (IBM, Armonk, NY).

## RESULTS

The antibacterial activity of each pulp therapy material was assessed using a disc diffusion susceptibility assay (CLSI, 2023) against the following cariogenic bacteria: *A. viscosus*, *L. acidophilus*, *L. rhamnosus*, and *S. mutans*, with saline as a negative control. FC exhibited the most significant antibacterial activity across all test cariogenic bacteria ($p$-value <0.05), with the highest efficacy obtained against *L. acidophilus* and *S. mutans*. The second best was ZOE, which exhibited comparable antibacterial activity against all four test bacteria. However, both materials demonstrated wider inhibition zones than the three MTA-based substances. All three MTA-based materials demonstrated comparable antibacterial activity against all four test cariogenic bacteria (Table 1).

## DISCUSSION

The study evaluated the antibacterial efficacy of five dental pulp therapy materials (FC, NeoMTA®, NeoPUTTY™, ProRoot® MTA, and ZOE) against four cariogenic bacteria (*A. viscosus*, *L. acidophilus*, *L. rhamnosus*, and *S. mutans*). All materials exhibited antibacterial activity based on the disc diffusion susceptibility assay.

Table 1

Disk diffusion susceptibility test of pulpotomy materials against selected cariogenic bacterial species

| Material | Inhibition zone (mm), mean ± SD | | | |
|---|---|---|---|---|
| | *Streptococcus mutans* | *Lactobacillus acidophilus* | *Actinomyces viscosus* | *Lactobacillus rhamnosus* |
| NeoPUTTY™ | 8.3 ± 0.7[a] | 12.3 ± 1.8[a] | 10.3 ± 2.6[a] | 9.1 ± 1.1[a] |
| NeoMTA® | 9.2 ± 0.5[a] | 11.2 ± 1.3[a] | 10.2 ± 1.2[a] | 8.2 ± 0.6[a] |
| ProRoot® MTA | 8.7 ± 0.6[a] | 13.4 ± 0.6[a] | 9.4 ± 1.6[a] | 8.3 ± 0.7[a] |
| Zinc oxide-eugenol | 18.3 ± 2.0[b] | 17.2 ± 1.8[b] | 15.1 ± 3.2[b] | 17.4 ± 1.3[b] |
| Formocresol | 40.2 ± 1.5[c] | 40.1 ± 2.3[c] | 23.0 ± 3.4[d] | 30.3 ± 1.3[e] |
| Saline (negative control) | 0 ± 0[f] | 0 ± 0[f] | 0 ± 0[f] | 0 ± 0[f] |

Note: Mean values sharing the same superscript are not significantly different from each other. Mean values with different superscripts are statistically significant ($p$-value <0.05, $n$ = 3). Disk diffusion susceptibility test was performed according to CLSI (2023) guidelines.

In agreement with previous studies, FC demonstrated the most potent bactericidal property, identifying it as the gold standard in pulpotomy (Noorollahian, 2008). FC efficacy is due to the presence of formaldehyde, which is well-known for its protein-denaturing and cytotoxic effects (Noorollahian, 2008). However, recent studies reporting adverse outcomes, such as internal resorption and chronic inflammation resulting from pulp irritation, have raised concerns regarding its cytotoxic and potentially carcinogenic properties, resulting in its limited clinical use (Noorollahian, 2008; Basir *et al*, 2019).

ZOE demonstrated significant antibacterial properties, though less potent than FC. The antimicrobial effect of ZOE is attributed to eugenol, which can disrupt bacterial cell walls and inhibit enzymatic activity, contributing to its antimicrobial effects (Basir *et al*, 2019). However, ZOE use is associated with pulp inflammation and internal resorption due to the release of free eugenol, causing moderate to severe inflammatory responses (Godhi *et al*, 2011; Singh *et al*, 2016; Basir *et al*, 2019). Although FC and ZOE exhibit superior antimicrobial properties, their cytotoxic effects limit their suitability for vital pulp therapy (Noorollahian, 2008; Chotvorrarak *et al*, 2017).

Only moderate antibacterial effects were observed with the test MTA-based materials. Previous research has suggested that the advantages of such materials in pulp therapy lie in their biocompatibility and sealing properties rather than antimicrobial activity (Pimenta *et al*, 2015; Shin *et al*, 2018). The antibacterial property of MTA is mainly due to the release of calcium hydroxide upon hydration, creating an alkaline environment that inhibits the bacterial growth of non- cariogenic microorganisms such as *Enterococcus faecalis* and *Staphylococcus aureus* (Noorollahian, 2008; Pimenta *et al*, 2015). However, its antibacterial efficacy is generally considered less potent compared

to materials like FC and ZOE (Mohammadi *et al*, 2012). Despite their weaker antimicrobial action compared to FC and ZOE, MTA-based materials remain a preferred pulp therapy due to their bioactivity, compatibility with pulp tissues and ability to promote the formation of hard tissues (Noorollahian, 2008; Basir *et al*, 2019).

In conclusion, this study demonstrated the superior anti-cariogenic bacterial efficacy of Buckley's formocresol (FC) and zinc oxide-eugenol (ZOE) compared to the mineral trioxide aggregate (MTA)-based dental pulp fillers NeoMTA®, NeoPUTTY™ and ProRoot® MTA. These findings highlight the trade-off between anti-cariogenic bacterial efficacy and biocompatibility/sealing property/hard tissue development properties of therapeutic substances used in pulpotomy. MTA-based materials offer a balanced approach by combining moderate antibacterial activity with bioactivity and biocompatibility in dental pulp therapy.

## ACKNOWLEDGEMENT

The author thanks the Dean of Scientific Research and the College of Dentistry Research Center at King Saud University, Saudi Arabia for funding the study, project no. FR0589.

## CONFLICT OF INTEREST DISCLOSURE

The author declares no conflict of interest.

## DATA AVAILABILITY STATEMENT

The study data are available from the author upon reasonable request.

## REFERENCES

American Academy of Pediatric Dentistry. Pulp Therapy for Primary and Immature Permanent Teeth, 2025 [cited 2025 Aug 28]. Available from: URL: https://www.aapd.org/globalassets/media/policies_guidelines/bp_pulptherapy.pdf

Basir L, Khanehmasjedi M, Khosravi

A, Ansarifar S. Investigating the antimicrobial activity of different root canal filling pastes in deciduous teeth. *Clin Cosmet Investig Dent* 2019; 11: 321-6.

Bjørndal L, Reit C, Bruun G, *et al*. Treatment of deep caries lesions in adults: randomized clinical trials comparing stepwise vs. direct complete excavation, and direct pulp capping vs. partial pulpotomy. *Eur J Oral Sci* 2010; 118(3): 290-7.

Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. *Int Endod J* 2019; 52(7): 949-73.

Bossù M, Iaculli F, Di Giorgio G, Salucci A, Polimeni A, Di Carlo S. Different pulp dressing materials for the pulpotomy of primary teeth: a systematic review of the literature. *J Clin Med* 2020; 9(3): 838.

Camp JH, Fuks AB. Pediatric endodontics: endodontic treatment for the primary and young permanent dentition. In: Cohen S, Hargreaves KM, editors. Pathways of the pulp. 9th ed. St. Louis, MO: Mosby; 2006. p. 822–53.

Chakraborty A, Dey B, Jana S. A nonconventional approach to formocresol pulpotomy. *Int J Clin Pediatr Dent* 2018; 11(6): 490-5.

Chotvorrarak K, Yanpiset K, Banomyong D, Srisatjaluk RL. In vitro antibacterial activity of oligomer-based and calcium silicate-based root canal sealers. *M Dent J* 2017; 37(2): 145-54.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

Coll JA, Seale NS, Vargas K, Marghalani AA, Al Shamali S, Graham L. Primary tooth vital pulp therapy: a systematic review and meta-analysis. *Pediatr Dent* 2017; 39(1): 16-123.

Godhi B, Sood PB, Sharma A. Effects of mineral trioxide aggregate and formocresol on vital pulp after pulpotomy of primary molars: an in vivo study. *Contemp Clin Dent* 2011; 2(4): 296-301.

Gonzalez-Lara A, Ruiz-Rodriguez MS, Pierdant-Perez M, Garrocho-Rangel JA, Pozos-Guillen AJ. Zinc oxide-eugenol pulpotomy in

primary teeth: a 24-month follow-up. *J Clin Pediatr Dent* 2016; 40(2): 107-12.

Güven EP, Taşlı PN, Yalvac ME, Sofiev N, Kayahan MB, Sahin F. *In vitro* comparison of induction capacity and biomineralization ability of mineral trioxide aggregate and a bioceramic root canal sealer. *Int Endod J* 2013; 46(12): 1173-82.

Katsamakis S, Slot DE, Van der Sluis LW, Van der Weijden F. Histological responses of the periodontium to MTA: a systematic review. *J Clin Periodontol* 2013; 40(4): 334-44.

Mohammadi Z, Giardino L, Palazzi F, Shalavi S. Antibacterial activity of a new mineral trioxide aggregate-based root canal sealer. *Int Dent J* 2012; 62(2): 70-3.

Naushad AM, Sunil JM, Kumar KSH, Sathar S, Mathews R, Gopal A. An *in vitro* study of probiotic activity exhibited by *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on oral isolates of *Streptococcus mutans* and *Candida albicans*. *Int J Res Med Sci* 2023; 11(6): 2062-7.

Noorollahian H. Comparison of mineral trioxide aggregate and formocresol as pulp medicaments for pulpotomies in primary molars. *Br Dent J* 2008; 204(11): E20.

Oliveira TM, Moretti ABS, Sakai VT, *et al*. Clinical, radiographic and histologic analysis of the effects of pulp capping materials used in pulpotomies of human primary teeth. *Eur Arch Paediatr Dent* 2013; 14(2): 65-71.

Patchett CL, Srinivasan V, Waterhouse PJ. Is there life after Buckley's formocresol? Part II - Development of a protocol for the management of extensive caries in the primary molar. *Int J Paediatr Dent* 2006; 16(3): 199-206.

Pimenta HC, Borges ÁH, Bandeca MC, *et al*. Antimicrobial activity of filling materials used in primary teeth pulpotomy. *J Int Oral Health* 2015; 7(4): 54-7.

Shin JH, Lee DY, Lee SH. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. *J Dent Sci* 2018; 13(1): 54-9.

Simancas-Pallares MA, Díaz-Caballero AJ, Luna-Ricardo LM. Mineral trioxide aggregate in primary teeth pulpotomy. A systematic literature

review. *Med Oral Patol Oral Cir Bucal* 2010; 15(6): e942-6.

Singh G, Gupta I, Elshamy FMM, Boreak N, Homeida HE. *In vitro* comparison of antibacterial properties of bioceramic-based sealer, resin-based sealer and zinc oxide eugenol based sealer and two mineral trioxide aggregates. *Eur J Dent* 2016; 10(3): 366-9.