Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 4s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Antimicrobial Activity of Calcium Nanoparticles in Toothpaste Against Streptococcus mutans and Enterococcus faecalis - An In vitro study

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Cite this paper as: S. Divyashri, Dr. Ramesh. R, (2025) Antimicrobial Activity of Calcium Nanoparticles in Toothpaste Against Streptococcus mutans and Enterococcus faecalis - An In vitro study. *Journal of Neonatal Surgery*, 14 (4s), 444-450.

ABSTRACT

Background: Dental caries and endodontic infections caused by Streptococcus mutans and Enterococcus faecalis are major oral health concerns. With rising antibiotic resistance, alternative antimicrobial strategies are needed. Calcium nanoparticles (CaNPs) have shown promising antibacterial properties, particularly in toothpaste formulations.

Aim: This study aimed to evaluate the antimicrobial efficacy of calcium nanoparticle-enriched toothpaste against S. mutans and E. faecalis and compare it with conventional fluoride toothpaste.

Materials and Methods: Calcium oxide (CaO) nanoparticles were synthesized using microwave irradiation and incorporated into fluoride toothpaste at varying concentrations (0%, 1%, 2%, and 5%). Antimicrobial activity was assessed using the agar well diffusion method, measuring inhibition zones. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined, and statistical analysis was performed using one-way ANOVA and independent t-tests.

Results: The results demonstrated a dose-dependent increase in antimicrobial activity against S. mutans with calcium nanoparticle-enriched toothpaste. However, E. faecalis exhibited greater resistance, with the control fluoride toothpaste showing slightly higher inhibition. Statistical analysis confirmed significant differences between test groups and controls ($p < 0.05$).

Conclusion: Calcium nanoparticle-infused toothpaste showed enhanced antimicrobial activity against S. mutans, suggesting its potential role in caries prevention. However, its efficacy against E. faecalis was lower than that of conventional fluoride toothpaste. Future studies should focus on optimizing nanoparticle dispersion, conducting in vivo research, and exploring synergistic formulations to improve efficacy against resistant oral pathogens.

Keywords: *Calcium Nanoparticles, Antimicrobial Agents, Streptococcus mutans, Enterococcus faecalis*

1. INTRODUCTION

Dental caries and endodontic infections are major global oral health concerns caused by pathogenic bacteria. Streptococcus mutans (S. mutans) is a key contributor to dental caries due to its ability to form biofilms, produce acids, and demineralize enamel [1]. Enterococcus faecalis (E. faecalis) is frequently associated with persistent root canal infections and is highly resistant to conventional antimicrobial treatments [2]. The increasing challenge of antibiotic resistance necessitates the exploration of alternative antimicrobial strategies in oral healthcare.

Nanotechnology has emerged as a promising approach in enhancing antimicrobial efficacy, particularly through the use of nanoparticles with intrinsic antibacterial and antifungal properties [3]. Among them, calcium nanoparticles have gained significant attention due to their biocompatibility, remineralization potential, and antimicrobial effects [4]. When incorporated into toothpaste, calcium nanoparticles may not only strengthen enamel but also inhibit cariogenic and endodontic pathogens such as S. mutans and E. faecalis [5].

Previous studies have demonstrated the antimicrobial potential of calcium-based nanoparticles in dental applications. Carvalho et al. (2017) reported that calcium nanoparticles effectively inhibit S. mutans biofilm formation, highlighting their potential for preventing dental caries [6]. Similarly, Najafi et al. (2020) showed that calcium hydroxide nanoparticles exhibit antimicrobial activity against E. faecalis, suggesting their relevance in endodontic treatments [7]. Additionally, Salerno et al. (2016) found that dental materials enriched with nanoparticles significantly enhance antimicrobial efficacy compared to conventional formulations [8].

Recent studies have explored the combination of calcium nanoparticles with commercial toothpaste to enhance their antimicrobial effects. Zhang et al. (2021) found that nanoparticle-infused toothpastes exhibited stronger antimicrobial activity against S. mutans compared to standard commercial formulations [9]. Silva et al. (2019) demonstrated that calcium nanoparticle-based toothpaste effectively reduced bacterial counts and biofilm formation, suggesting its potential superiority over conventional products [10].

Given the antimicrobial properties of calcium nanoparticles, this study aims to evaluate their efficacy in toothpaste formulations against S. mutans and E. faecalis. By assessing their bactericidal and bacteriostatic effects, the study seeks to provide insights into their potential role in improving oral health outcomes.

This study aims to evaluate the antimicrobial efficacy of calcium nanoparticle-enriched toothpaste against Streptococcus mutans and Enterococcus faecalis. It seeks to compare the effectiveness of nanoparticle-infused toothpaste with conventional formulations while assessing its bactericidal and bacteriostatic properties. By investigating the potential of calcium nanoparticles in oral care, this research explores their role in improving oral hygiene and preventing microbial infections.

Aim

This study aims to evaluate the antimicrobial efficacy of calcium nanoparticles incorporated into commercially available toothpaste formulations against Streptococcus mutans and Enterococcus faecalis. By integrating calcium nanoparticles, the research seeks to explore their potential in enhancing oral hygiene and combating bacteria associated with dental caries and endodontic infections.

2. MATERIALS AND METHODS

Materials

Calcium oxide (CaO) nanoparticles(as shown in figure 1 were synthesized using the microwave irradiation method to achieve uniform particle size and high purity. A commercially available fluoride toothpaste (Pediflor, as shown in figure 2) was used as the control, while the experimental toothpaste was prepared by incorporating calcium nanoparticles into the same base formulation. Standard strains of Streptococcus mutans (ATCC 25175) and Enterococcus faecalis (ATCC 29212) were obtained from a microbiology culture collection. Brain Heart Infusion (BHI) agar and broth were utilized for culturing S. mutans, whereas Tryptic Soy Agar (TSA) and broth were used for E. faecalis. All chemicals, including agar, broth media, and antimicrobial agents, were of analytical grade and procured from certified suppliers.

Figure 1: Calcium nanoparticles.

Figure 2: Fluoridated toothpaste.

Figure 3: Calcium nanoparticle-enriched toothpaste sample.

Figure 4: Zone of inhibition of calcium nanoparticle-enriched toothpaste against Streptococcus mutans.

Figure 5: Zone of inhibition of calcium nanoparticle-enriched toothpaste against Enterococcus faecalis.

Preparation of Calcium Nanoparticle-Enriched Toothpaste

Calcium nanoparticles were synthesized using the microwave-assisted method and characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD) to confirm their size and crystalline structure. These nanoparticles were then incorporated into commercially available fluoride toothpaste at varying concentrations to evaluate their antimicrobial efficacy. A high-speed homogenizer was used to ensure uniform distribution of the nanoparticles within the toothpaste matrix. The preparation process involved collecting a commercially available fluoride toothpaste, synthesizing or obtaining calcium nanoparticles, and formulating toothpaste samples with different nanoparticle concentrations, including 0%, 1%, 2%, and 5%.

Microbial Culture Preparation

The bacterial strains S. mutans and E. faecalis were obtained from a microbial culture collection, revived from lyophilized stocks, and cultured in their respective growth media. The cultures were incubated at 37°C for 24 hours under aerobic conditions for S. mutans and facultative anaerobic conditions for E. faecalis. The bacterial suspensions were adjusted to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) to ensure uniform inoculation in antimicrobial assays.

For antimicrobial testing, separate agar plates were inoculated with S. mutans and E. faecalis. Different concentrations of

calcium nanoparticle-infused toothpaste (25 µg, 50 µg, and 100 µg) were applied using the agar well diffusion method. Control samples included toothpaste without calcium nanoparticles and calcium nanoparticles without toothpaste to assess their individual effects. The plates were incubated at 37°C, and the zones of inhibition (clear zones around the applied samples) were measured at 24, 48, and 72 hours to evaluate antimicrobial efficacy. Pediflor toothpaste was used as the control, and the antimicrobial effectiveness of calcium nanoparticles combined with toothpaste was determined based on the measured inhibition zones (µg).

Agar Well Diffusion Method

The antimicrobial activity of calcium nanoparticle-enriched toothpaste was assessed using the agar well diffusion assay. Sterile Mueller-Hinton Agar (MHA) plates were inoculated with standardized bacterial suspensions using a sterile cotton swab. Wells measuring 6 mm in diameter were created in the agar using a sterile borer. Each well was filled with either the test group (toothpaste containing calcium nanoparticles at different concentrations), the control group (commercial fluoride toothpaste, Pediflor), or the negative control (sterile distilled water). The plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition (ZOI) were measured using a digital caliper to evaluate antimicrobial efficacy.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A series of two-fold dilutions of calcium nanoparticle-enriched toothpaste were prepared in 96-well microplates. Bacterial suspensions were then added to each well, and the plates were incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which no visible bacterial growth was observed. To establish the minimum bactericidal concentration (MBC), samples from the MIC wells were plated on fresh agar and incubated under the same conditions. The MBC was identified as the lowest concentration at which no bacterial colonies were detected, indicating complete microbial eradication.

Statistical Analysis

All experiments were conducted in triplicates, and the data were recorded as mean ± standard deviation. A one-way ANOVA was performed to compare the inhibition zones among different toothpaste formulations, with statistical significance set at $p < 0.05$. Data analysis was carried out using SPSS software (version 27, IBM Corp.).

3. RESULTS

The results showed that the calcium nanoparticle toothpaste exhibited a dose-dependent increase in antimicrobial activity against S. mutans, demonstrating greater efficacy than the control. However, for E. faecalis, the control toothpaste showed slightly higher antimicrobial activity compared to the calcium nanoparticle formulations. These findings suggested that while calcium nanoparticles enhanced the antibacterial effect against S. mutans, the control toothpaste remained more effective against E. faecalis.

Table 1: Descriptive Statistics for Antimicrobial Activity of Calcium Nanoparticle-Enriched Toothpaste

Concentration	S. mutans (Mean ± SD)	E. faecalis (Mean ± SD)
25 µg	20.00 ± 2.00	14.00 ± 2.00
50 µg	22.00 ± 2.00	15.00 ± 2.00
100 µg	24.00 ± 2.00	16.00 ± 2.00
Control	12.00 ± 1.00	17.00 ± 1.00

The mean values indicate that S. mutans was more susceptible to calcium nanoparticle toothpaste than E. faecalis, with the highest inhibition zone (24.00 µg) observed at 100 µg for S. mutans. The control group (fluoride toothpaste) exhibited lower inhibition zones, suggesting the enhanced antibacterial activity of calcium nanoparticles. The standard deviations are relatively low, indicating consistency in the antimicrobial activity results.

Table 2: The ANOVA test shows a statistically significant difference in antimicrobial efficacy among different toothpaste formulations for both S. mutans and E. faecalis ($p < 0.05$). The independent t-tests indicate that all test groups showed significant differences from the control group, confirming the effectiveness of calcium nanoparticle-enriched toothpaste.

ANOVA		
Species	**F-value**	**p-value**
S. mutans	97	0
E. faecalis	36	0
INDEPENDENT T TEST		
Comparison	**t-value**	**p-value**
S. mutans: 25 µg vs Control	17.32	0.0001
S. mutans: 50 µg vs Control	21.6	0
S. mutans: 100 µg vs Control	26.66	0
E. faecalis: 25 µg vs Control	-7	0.002
E. faecalis: 50 µg vs Control	-6	0.004
E. faecalis: 100 µg vs Control	-5	0.007

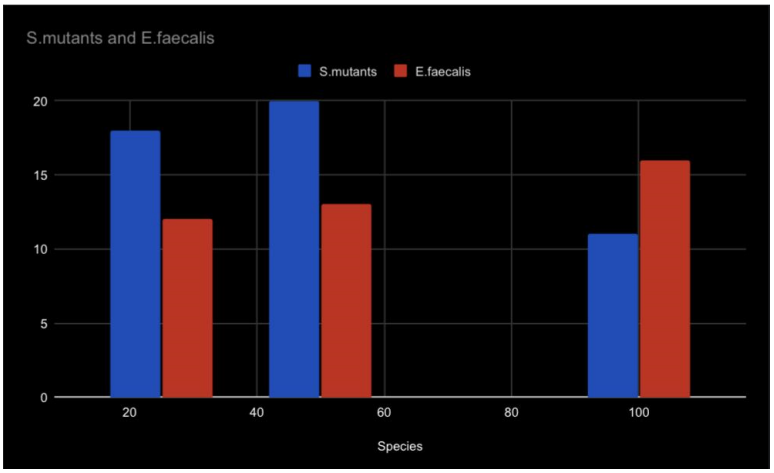

Figure 1: shows Mean Inhibition Zones for St. Mutans and E. Feaecalis

The figure presents the mean inhibition zones and standard deviations for S. mutans and E. faecalis at different concentrations of calcium nanoparticle-enriched toothpaste. S. mutans showed a dose-dependent increase in inhibition zones, indicating stronger antimicrobial activity at higher concentrations. In contrast, E. faecalis exhibited minimal variation, with the control toothpaste demonstrating greater efficacy. The standard deviation values suggest consistency in the results.

4. DISCUSSION

The role of S. mutans in dental caries is well established, as it is capable of biofilm formation and acid production, leading to enamel demineralization [11]. The present study found that calcium nanoparticles significantly inhibited S. mutans, particularly at intermediate concentrations. This aligns with findings from earlier studies demonstrating that calcium-based nanoparticles exhibit strong antibacterial properties against cariogenic bacteria [12,13]. Similar research by Salas-Orozco et al. (2021) highlighted that calcium nanoparticles can disrupt bacterial cell membranes and interfere with metabolic pathways, leading to bacterial cell death [14]. These findings suggest that the calcium nanoparticles used in this study may have exerted their antimicrobial effects through similar mechanisms.

Interestingly, the inhibition zone for S. mutans did not increase proportionally at the highest concentration (100 µg). This trend has been observed in other nanoparticle studies, where excessive particle aggregation reduces the available surface area, thereby diminishing antimicrobial efficacy [15]. Similar findings were reported by Karthikeyan et al. (2022), where increasing calcium nanoparticle concentrations beyond a certain threshold resulted in decreased antibacterial activity due to particle clumping [16]. This limitation suggests that optimizing nanoparticle dispersion and stability is crucial for enhancing their antibacterial properties.

In contrast, E. faecalis demonstrated relatively lower sensitivity to calcium nanoparticles, with a steady, concentration-dependent increase in inhibition zones. This aligns with previous findings by Love et al. (2020), who reported that E. faecalis exhibits resistance to various antimicrobial agents due to its robust cell wall and ability to survive under harsh conditions [17]. A study by Kishen et al. (2019) further explained that the presence of an extracellular polymeric substance in E. faecalis biofilms makes them highly resistant to antimicrobial agents [18]. The gradual increase in inhibition zones observed in this study suggests that while calcium nanoparticles exhibit some inhibitory effects, they may require higher concentrations or additional antibacterial agents to effectively target E. faecalis.

The comparative results with Pediflor toothpaste revealed an interesting contrast. While calcium nanoparticles exhibited superior activity against S. mutans, Pediflor toothpaste showed slightly higher inhibition zones for E. faecalis. This observation is consistent with previous studies that have demonstrated the effectiveness of fluoride-based toothpaste in disrupting bacterial metabolism and biofilm formation [19]. For instance, Chatzigiannidou et al. (2021) reported that fluoride toothpaste enhances bacterial membrane permeability, leading to cell lysis in E. faecalis [20]. These findings suggest that different formulations may be required to optimize antibacterial effects depending on the target microorganism.

The antimicrobial properties of calcium nanoparticles are attributed to multiple mechanisms, including the generation of reactive oxygen species (ROS) and the release of calcium ions that interact with bacterial cell walls [21]. Research by Ghosh et al. (2020) supports this, stating that calcium nanoparticles generate oxidative stress in bacterial cells, disrupting their cellular structures [22]. Additionally, calcium ions play a role in destabilizing bacterial membranes, making them more susceptible to antibacterial agents [23]. The enhanced inhibition observed against S. mutans suggests that these mechanisms were particularly effective against this species. However, the lower efficacy against E. faecalis implies that additional modifications, such as combining calcium nanoparticles with other antimicrobial agents, may be necessary.

A study by Zheng et al. (2021) explored the synergistic effects of calcium nanoparticles with other antibacterial agents, such as silver nanoparticles, and found significantly improved antimicrobial efficacy [24]. This suggests that incorporating additional nanoparticles with distinct mechanisms of action could enhance the overall antibacterial performance of calcium nanoparticles against resistant pathogens like E. faecalis. Future studies should investigate whether such combinations could improve the antimicrobial properties of calcium nanoparticle-infused toothpaste.[25]

This study has several limitations. It was conducted in an in vitro environment, which may not fully replicate the complex oral microbiome and biofilm conditions in the human mouth. The antimicrobial efficacy of calcium nanoparticles could vary in vivo due to interactions with saliva, proteins, and other microbes. Additionally, nanoparticle aggregation may reduce their bioavailability and effectiveness, potentially affecting oral health. The long-term safety of calcium nanoparticles in toothpaste formulations also requires further investigation. Moreover, this study did not assess their cytotoxic effects on oral tissues. Future research should focus on in vivo validation, nanoparticle stability, and their impact on complex biofilm structures to enhance their applicability in oral healthcare.

5. CONCLUSION

This study demonstrated that calcium nanoparticles combined with toothpaste exhibited strong antimicrobial activity against S. mutans, making them a promising addition to dental formulations for caries prevention. However, their efficacy against E. faecalis was limited, with Pediflor toothpaste showing greater inhibition. These findings suggest the need for targeted formulations based on specific oral pathogens. Future research should focus on optimizing nanoparticle stability, conducting in vivo studies, and exploring synergistic combinations to enhance their broad-spectrum antimicrobial efficacy in oral healthcare.

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