ORIGINAL ARTICLE

International Journal of Dental Materials (ISSN: 2582-2209 (Online))
DOI: http://dx.doi.org/10.37983/IJDM.2025.7302

Antibacterial Efficacy of Silver Diamine Fluoride and 3M ESPE Clinpro™ Tooth Crème Against *Streptococcus mutans*: A Comparative *in vitro* study

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Article History
Received: 22nd July 2025
Accepted: 3rd September 2025
Published: 30th September 2025

Abstract

Background: Remineralizing agents such as Silver Diamine Fluoride (SDF) and 3M ESPE™ Clinpro Tooth Crème have gained attention for their potential antimicrobial and enamel-strengthening properties. While fluoride-based therapies are widely used, there is limited comparative data on the antimicrobial efficacy of these agents.

Aim: To evaluate and compare the antimicrobial effects of remineralizing agents in inhibiting *S. mutans* growth.

Materials and methods: This *in vitro* study was conducted following ethical approval. The study involved 56 samples divided into four groups: Group I (SDF), Group II (3M ESPE™ Clinpro Tooth Crème), Group III (2% Chlorhexidine - Positive Control), and Group IV (Distilled Water - Negative Control). The antimicrobial efficacy of each agent was evaluated using the Agar well diffusion method, with the Zone of Inhibition (ZOI) measured in millimetres. Statistical analysis was performed to determine the significance of differences between the groups.

Results: The results demonstrated that SDF exhibited the highest antimicrobial efficacy, with a significantly larger zone of inhibition compared to 3M ESPE™ Clinpro Tooth Crème.

Conclusion: The findings suggest that SDF is more effective in bacterial inhibition compared to 3M ESPE™ Clinpro Tooth Crème. The dual action of remineralization and antimicrobial activity highlights SDF as a promising agent for caries prevention and management. Further *in vivo* studies are required to validate these results in clinical settings.

Keywords: Antibacterial efficacy, Clinpro Tooth Crème, Remineralizing agents, Silver Diamine Fluoride.

Cite this article as: Shri Kavya A, Amitha HA, Aniyoor S, Prabha MS, Shetty NJ. Antibacterial Efficacy of Silver Diamine Fluoride and 3M ESPE ClinproTM Tooth Crème Against Streptococcus mutans: A Comparative *in vitro* study. **Int J Dent Mater. 2025;7(3):76-83.**

1. Introduction

Dental caries is one of the most prevalent chronic infectious diseases affecting children and adults worldwide. It is a multifactorial disease resulting from the interaction of cariogenic bacteria, fermentable carbohydrates, and host factors, including saliva and tooth structure [1]. *Streptococcus mutans* (*S. mutans*) plays a crucial role in biofilm formation and acid production, which leads to enamel demineralization and the formation of cavities [2].

The caries process is governed by a continuous cycle of demineralization and remineralization. Demineralization occurs when acids produced by bacteria dissolve hydroxyapatite crystals in enamel, while remineralization restores lost minerals with the help of calcium, phosphate, and fluoride available in the saliva. Fluoride plays a crucial role in enhancing remineralization by forming fluorapatite, a more acid-resistant mineral [3,4].

The prevention and management of dental caries have evolved significantly, focusing on enhancing remineralization and inhibiting bacterial activity. Fluoride-based interventions, such as Silver Diamine Fluoride (SDF) and Functionalised tricalcium phosphate-based 3M ESPE™ Clinpro tooth crème remineralizing agents, have gained attention for their ability to prevent further tooth decay and combat the early demineralisation stage, respectively.

Silver Diamine Fluoride (SDF) is a topical solution widely used in paediatric dentistry for caries arrest and prevention. It contains silver, which has potent antibacterial properties, and also fluoride, which enhances remineralization [5,6]. 3M ESPE™ Clinpro Tooth Crème is a remineralizing agent containing functionalised tricalcium phosphate (fTCP) and sodium fluoride (mention concentration). The functionalized tricalcium phosphate (fTCP) technology stabilises calcium and phosphate ions, ensuring effective enamel repair [7].

Several studies have investigated the antimicrobial properties of SDF, consistently demonstrating its strong antibacterial effects. Research has shown that SDF significantly reduces bacterial viability and biofilm formation. Studies by Zhi *et al.* (2012) [6] found that SDF effectively inhibited *S. mutans* growth, reduced bacterial adhesion, and disrupted mature biofilms. Additionally, clinical studies, such as those by Chu *et al*. (2014) [8] and Gao *et al*. (2016) [9], reported that SDF-treated lesions exhibited arrested caries progression and sustained bacterial inhibition.

Studies on fTCP technology have suggested that it not only promotes remineralization but also exhibits antibacterial effects. Research by Karlinsey *et al*. (2009) [10] demonstrated that fTCP combined with fluoride significantly inhibited *S. mutans* biofilm formation and acid production. Similarly, Shen *et al*. (2011) reported reduced bacterial colonisation on fluoride-treated enamel surfaces due to the synergistic action of fTCP and fluoride [11].

Given the increasing prevalence of dental caries, effective preventive and therapeutic strategies are essential. While both SDF and 3M ESPE™ Clinpro have shown promise, their relative antibacterial effectiveness against *S. mutans* remains an area of ongoing research. This study aims to compare the antibacterial efficacy of SDF and 3M ESPE™ Clinpro against *S. mutans* in an *in vitro* microbiological model.

2. Materials and methods

The study was an in vitro microbiological study. Ethical clearance (KIMS/IEC/D238/D/2023) for this study was obtained from the Institutional Research and Ethical Committee, Kempe Gowda Institute of Medical Sciences, Bengaluru, Karnataka, India.

This in vitro study evaluated three remineralizing agents: Kids-e-SDF (SDF) (Kids-e-Dental), 3M ESPE™ Clinpro, and 2% w/w Chlorhexidine (CHX). Chlorohexidine served as the positive control, while distilled water was used as a negative control. A total of 56 samples were prepared with 14 (n=14) for each material, Group I - Kids E SDF (Kids-e-Dental), composed of 38% Silver diamine fluoride (SDF), Group II - 3M ESPE™ Clinpro tooth crème, composed of 0.21% w/w sodium fluoride, tricalcium phosphate, Group III – Chlorhexidine 2% w/w (CHX) (positive control) and Group IV - distilled water (negative control). A convenience sampling method was employed, and all the experiments were performed in triplicate to account for possible loss and to ensure consistency in results.

The *Streptococcus mutans* (MTCC 497) strain was procured in a freeze-dried form from the MTCC repository and stored at 4°C until use. After opening the vial, sterile water was added, and the contents were mixed thoroughly to rehydrate the bacterial culture. For activation, the strain, the Luria Bertani (LB) broth was used, and incubated for one hour.

2.1 Culture media for bacterial growth

About 30 mL of LB broth was prepared by adding tr yptone 0.3 gm, sodium chloride 0.3 gm, yeast extrac t 0.18 gm, and distilled water 30 mL. The culture m edia were then autoclaved at 121°C for 15 minutes. The *S. mutans* strain (*MTCC 497*) was inoculated an d incubated at 37°C for 24 hours. Later, 5 mL of bro th culture was centrifuged at 6000 rpm for 10 minu tes, the supernatant was discarded, and the pellets were dissolved in 1% saline. Following activation, a sterile cotton swab was dipped into the LB broth co ntaining the bacteria and used to streak *S. mutans* o nto blood agar and sheep blood agar plates. These p lates were incubated at 37°C for 24 hours, allowing for visible bacterial growth to develop.

2.2 Sample preparation

The samples were prepared by adding dimethyl sulfoxide (DMSO) to the respective study groups. DMSO acted as a solvent for the dissolution of study materials.

About 10 mg of 3M ESPE™ Clinpro tooth crème was dissolved in 1 mL of DMSO, and 10 µL (100 µg) was pipetted out and made up to 50 µL by adding DMSO. Also, 10 µL of SDF, 3M ESPE™ Clinpro tooth crème, CHX, and distilled water were made up to 50 µL by following the same method (Figure 1).

2.3 Plating for MIC against organisms

Approximately 25 mL of LB agar was added to the sterilised Petri plates and left to solidify. 200 µL of the prepared inoculum *of S. mutans* was applied to agar plates and spread thoroughly using a plate spreader. Five wells measuring about 0.6 cm were made in each plate using the borer (Figure 2), and 50 µL of the prepared sample was loaded into the corresponding wells; 50 µL of DMSO was loaded in the middle well as a check (Figure 3). The Petri plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was recorded in millimetres (mm).

2.4 Statistical analyses

The data was collected, coded and fed into SPSS (IBM version 23.0, USA) for statistical analysis. The descriptive statistics included the mean and standard deviation. The inferential statistics included a one-way ANOVA test followed by post-hoc Tukey's test for comparison of ZOI among the 4 groups. The level of significance was set at 0.05 at a 95% confidence interval.

3. Results

The antibacterial efficacy was determined using the agar well diffusion method, with inhibition zones measured after 24 hours of incubation. The assessment was conducted using 14 different plates to ensure accuracy and reproducibility of the results.

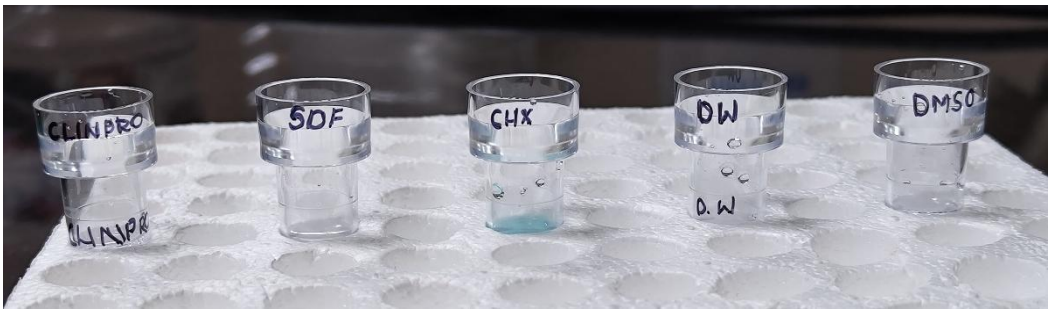

Figure 1. Different test solutions used in the study, including Clinpro, Silver Diamine Fluoride (SDF), Chlorhexidine (CHX), Distilled Water (DW), and Dimethyl Sulfoxide (DMSO).

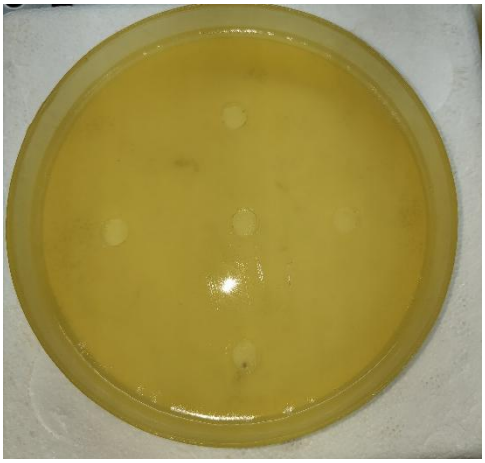

Figure 2. Wells punched in agar plates using a sterile borer (0.6 cm diameter).

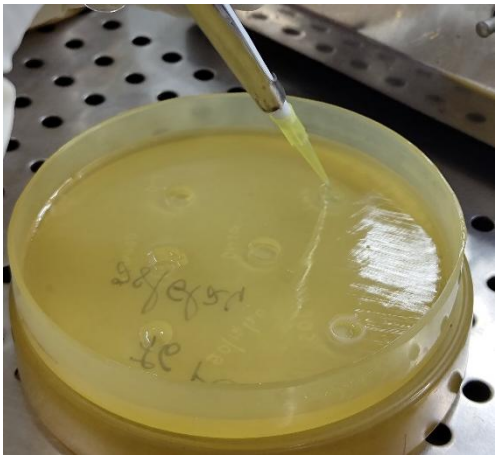

Figure 3. Loading 50 µL of each sample into its corresponding wells.

Table 1. Comparison of mean zones of inhibition among remineralizing agents (One-way ANOVA).

Groups	Mean ± SD#	F-value	Significance (p-value)
SDF	23.857 ± 3.255	634.284	0.000*
3M ESPE™ Clinpro	0.000 ± 0.000		
CHX	32.214 ± 3.683		
Distilled water	0.000 ± 0.000		

#Standard deviation, and *statistically significant.

Table 2. Pairwise comparison of remineralizing agents (post-hoc Tukey's test)

Groups		Mean ± Standard Error	Significance (p-Value)
SDF	3M ESPE™ Clinpro	23.857 ± 0.93	0.000*
	CHX	8.357 ± 0.93	0.000*
	Distilled water	23.857 ± 0.93	0.000*
3M ESPE™ Clinpro	CHX	32.214 ± 0.93	0.000*
	Distilled water	0.000 ± 0.93	1.000
CHX	Distilled water	32.214 ± 0.93	0.000*

*statistically significant.

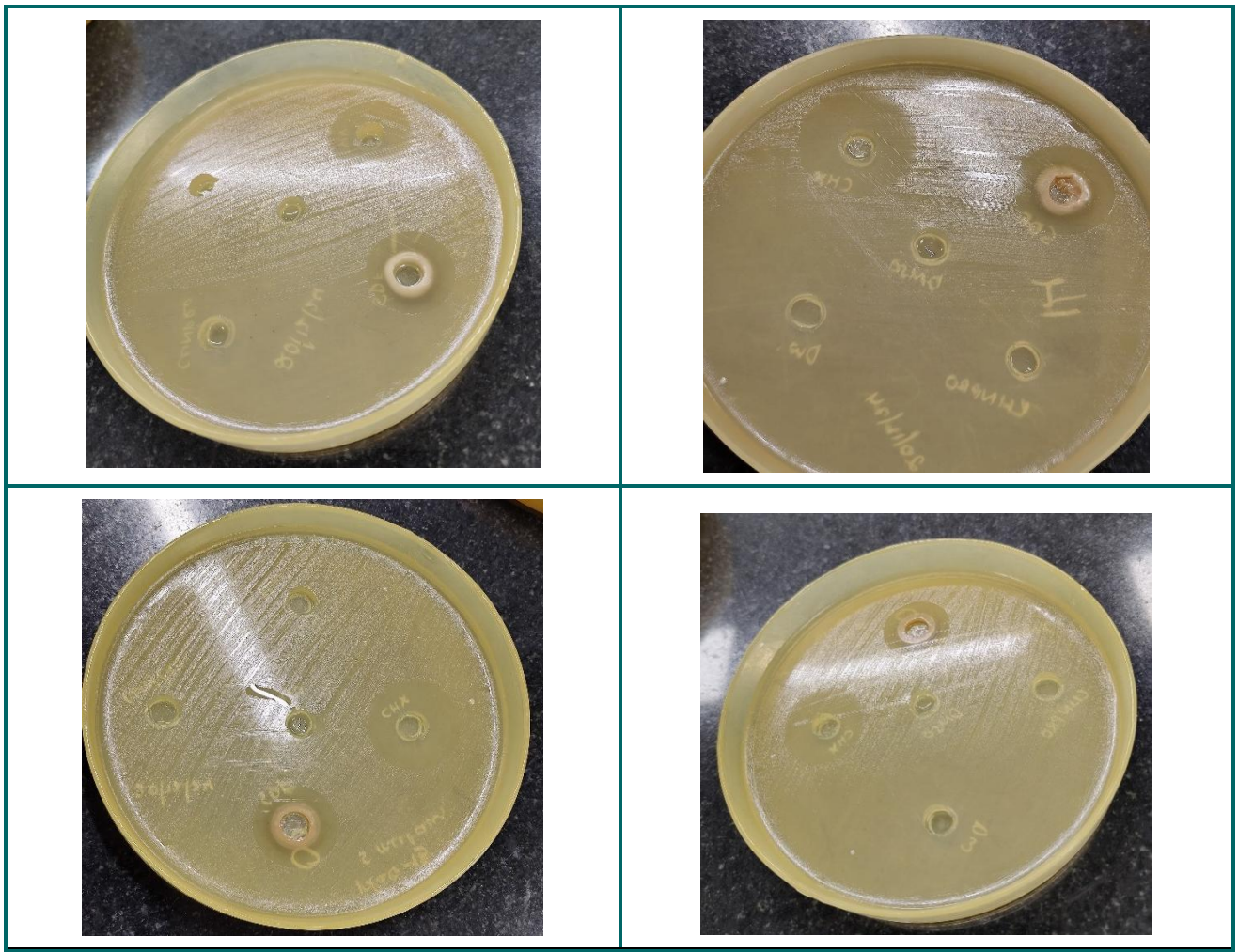

Figure 4. The zones of inhibition on LB agar plates after 24 hours of incubation.

Table 1 presents the mean zones of inhibition (ZOI) for the tested groups. The highest mean ZOI (Figure 4) was recorded with the CHX group (32.214 ± 3.683 mm), followed by the Kids e-SDF group (23.857 ± 3.255 mm). In contrast, 3M ESPE™ Clinpro demonstrated no detectable inhibition (0.000 mm), indicating a lack of antibacterial activity against *S. mutans*. As expected, distilled water, used as a negative control, also produced no inhibition zones. One-way ANOVA revealed a significant difference among the groups, with an F-value of 634.284 and a p-value of 0.000.

Post-hoc analysis (Table 2) revealed that the SDF exhibited significant differences when compared with 3M ESPE™ Clinpro, CHX, and distilled water (p=0.000). Similarly, CHX differed significantly from SDF, 3M ESPE™ Clinpro, and distilled water (p=0.000). In contrast, no significant difference was observed between 3M ESPE™ Clinpro and distilled water (p=1.000). These findings highlight the superior antimicrobial efficacy of SDF and CHX, suggesting their potential clinical utility in managing S. mutans-associated caries, whereas 3M ESPE™ Clinpro may primarily serve as a remineralizing adjunct rather than an antibacterial agent.

4. Discussion

Dental caries remains a significant global health concern, with *Streptococcus mutans* playing a pivotal role in its pathogenesis. Various remineralizing agents have been developed to combat this issue [12]. This study aimed to compare the antibacterial efficacy of the remineralising agents against *S. mutans* in an in vitro microbiological setting.

The findings of the current study indicate that SDF exhibited a superior antibacterial effect against *S. mutans* compared to 3M ESPE™ Clinpro tooth creme. This result aligns with previous studies that have demonstrated the potent antimicrobial properties of silver ions in SDF, which disrupt bacterial cell walls, interfere with DNA replication, and ultimately lead to bacterial cell death [13]. Silver ions exhibit oligodynamic action, meaning they exert their antimicrobial effects even at low concentrations, making SDF an effective long-term agent for caries control [14]. Furthermore, fluoride present in SDF enhances remineralization by forming fluorapatite, which is more resistant to acid dissolution, contributing to its dual role in both caries prevention and arrest [15].

In contrast, 3M ESPE™ Clinpro tooth crème, containing Functionalised tricalcium phosphate (fTCP) and fluoride, did not show antibacterial activity. fTCP acts as a bioavailable calcium and phosphate source, promoting enamel remineralization by facilitating hydroxyapatite formation. However, its antimicrobial properties are relatively weaker compared to the bactericidal action of silver in SDF [10]. This finding aligns with reports indicating that fluoride-based remineralizing agents primarily reduce bacterial adhesion and metabolic activity, whereas their direct bactericidal action is comparatively weaker than that of silver-based compounds. Additionally, the fluoride release from 3M ESPE™ Clinpro tooth crème is gradual, which may contribute to sustained but lower antibacterial activity [16].

Another critical factor influencing the antibacterial effectiveness of these agents is their mode of application. SDF is typically applied in a liquid form, allowing deeper penetration into dentinal tubules, which may enhance its long-term antimicrobial effect. This observation is corroborated by Li Y *et al.* (2019), who reported that SDF-treated dentin demonstrated silver particle penetration up to 200 µm, indicating prolonged antibacterial protection [17]. Similarly, Zhao *et al*. (2018) [18] highlighted that the silver ions in SDF occlude dentinal tubules and establish a barrier that prevents bacterial reinvasion. In contrast, 3M ESPE™ Clinpro Tooth Crème, formulated as a paste, requires sustained contact time for significant action. Karlinsey *et al*. (2009) [7] further support this by stating that the functionalized tricalcium phosphate (fTCP) component of 3M ESPE™ Clinpro requires enzymatic activation by saliva to release calcium and phosphate ions. Consequently, its effectiveness may vary based on individual salivary flow and composition. Hicks *et al*. (2004) also emphasised that fluoride-containing pastes generally require repeated and prolonged exposure to maintain their antibacterial potential, particularly in high-risk caries cases [19].

3M ESPE™ Clinpro Tooth Crème contains 0.21% sodium fluoride, delivering 950 ppm fluoride ions [10]. Incorporating stannous fluoride into remineralizing agents offers notable advantages over sodium fluoride, particularly regarding antibacterial properties. Stannous fluoride not only aids in enamel remineralization but also possesses significant antibacterial effects. The tin ions in stannous fluoride exhibit both bacteriostatic and bactericidal activities, effectively inhibiting the growth of and killing bacteria like *Streptococcus*

mutans, a primary contributor to dental caries [20]. According to Rosen *et al*. (1978), stannous fluoride significantly inhibited the growth of *Streptococcus mutans* by interfering with bacterial glycolysis and promoting substantial tin uptake into the cells, a property not observed with sodium fluoride [21]. Similarly, Dibdin and Shellis (1984) demonstrated that stannous fluoride showed stronger suppression of *S. mutans* growth and acid production compared to sodium fluoride in a controlled biofilm model [22]. Furthermore, a comparative study by Tseng *et al.* (1992) found that a 0.4% SnF_2 formulation exhibited antibacterial effects comparable to 0.12% chlorhexidine, a gold-standard antimicrobial agent [23]. These findings collectively highlight the superior antibacterial efficacy of stannous fluoride, reinforcing its role not only in remineralization but also in long-term caries prevention.

Silver diamine fluoride (SDF) has demonstrated broad-spectrum antimicrobial activity extending beyond *Streptococcus mutans*, effectively targeting other cariogenic bacteria such as Lactobacillus species. In a study by Mei *et al.* (2012), the application of 38% SDF significantly inhibited the growth of a multi-species biofilm comprising *S. mutans, S. sobrinus, Lactobacillus acidophilus, L. rhamnosus, and Actinomyces naeslundii* on dentin surfaces [13]. The treated group exhibited markedly lower colony-forming units (CFUs) and reduced biofilm formation compared to the control group, indicating SDF's efficacy in reducing the overall microbial load in the oral cavity [13]. 3M ESPE™ Clinpro tooth crème, however, primarily functions by promoting remineralization rather than directly eradicating bacterial colonies, making it more suitable for patients with low to moderate caries risk rather than those with extensive active lesions.

While silver diamine fluoride (SDF) is effective in arresting dental caries, studies have raised concerns regarding its cytotoxicity to pulpal cells. Fancher *et al*. (2019) reported that SDF-treated hydroxyapatite discs remained cytotoxic to human gingival fibroblasts even after 9 weeks of rinsing, indicating prolonged cytotoxic effects [24]. Similarly, a study by Kim *et al*. (2021) demonstrated that SDF adversely affected the viability and morphology of pulpal-like cells, although the addition of reduced glutathione mitigated these toxic effects [25]. The long-term impact of repeated SDF applications on the oral microbiome remains an area of ongoing research. While SDF exhibits broad-spectrum antimicrobial activity, its effects on the balance of the oral microbial community over extended periods are not fully understood. Additionally, although the potential for bacterial resistance to silver-based compounds is currently minimal, continuous monitoring is essential to detect any emerging resistance patterns. 3M ESPE™ Clinpro tooth crème, while effective in promoting remineralization, may not provide sufficient bacterial eradication in cases of aggressive caries progression, necessitating adjunctive antimicrobial therapies.

Our study has certain limitations, including its in vitro nature, which may not fully replicate the complex oral environment. Saliva, biofilm formation, and patient-specific factors such as dietary habits and oral hygiene practices can significantly influence the efficacy of these agents in vivo. Future studies incorporating in vivo models and clinical trials are necessary to validate these findings and assess the long-term effects of these agents on caries prevention and arrest. Additionally, further research should explore the potential of combining 3M ESPE™ Clinpro with other remineralizing agents to optimise both antibacterial and remineralization properties without significant drawbacks. Another aspect that warrants further exploration is the potential systemic absorption of silver from SDF applications. Comparative cost-effectiveness analysis of these agents should also be considered, as SDF is generally more affordable compared to advanced remineralization systems like 3M ESPE™ Clinpro tooth crème. This economic factor may influence the accessibility and widespread adoption of these treatments in resource-limited settings.

Overall, our results suggest SDF demonstrates a more pronounced antimicrobial effect, reinforcing its potential as a superior agent in caries management. However, while selecting a remineralizing agent, one should also consider patient-specific factors, including aesthetic concerns due to SDF-induced discolouration, fluoride exposure, and caries risk assessment. The integration of these agents into a comprehensive caries management strategy should be tailored to individual patient needs to optimise clinical outcomes. Further research is needed to explore combination therapies and alternative formulations that can maximise both antibacterial and remineralizing benefits while minimising potential drawbacks.

This study was limited its in vitro design and controlled conditions, which may not fully replicate

the complex oral environment. While stannous fluoride has shown antibacterial effects comparable to chlorohexidine in earlier studies, its efficacy was not evaluated in this study, and direct comparisons with SDF remain scarce. Future research should include well-designed in vivo and clinical studies comparing agents such as tricalcium phosphate, stannous fluoride, and SDF to establish their relative effectiveness in caries prevention. Furthermore, modifications to 3M ESPE™ Clinpro Tooth Crème, such as incorporating antimicrobial components, or hybrid strategies that combine reminerlizing and antibacterial actions, may offer more comprehensive caries management, particularly for high-risk populations.

Future research should include well-designed in vivo and clinical studies comparing agents such as tri-calcium phosphate, stannous fluoride, and SDF to establish their relative effectiveness in caries prevention. Furthermore, modifications to 3M ESPE Clinpro Tooth Crème—such as incorporating antimicrobial components—or hybrid strategies that combine remineralizing and antibacterial actions may offer more comprehensive caries management, particularly for high-risk populations.

5. Conclusion

This study highlights the superior antibacterial activity of silver diamine fluoride (SDF) against *Streptococcus mutans*, attributed to the synergistic effects of silver ions and fluoride. In contrast, 3M ESPE™ Clinpro Tooth Crème, based on functionalised tricalcium phosphate and fluoride, primarily supports remineralization with limited antibacterial action. While SDF offers a dual-action approach for caries arrest, 3M ESPE™ Clinpro may serve better as a supplementary remineralizing agent. Future in vivo studies, formulation improvements, and hybrid strategies combining remineralizing and antimicrobial components are warranted to optimise caries management, especially in high-risk groups.

Conflicts of interest: The Authors declared no conflicts of interest.
Financial support: None.

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