Nano-Silver Fluoride in Preventive Dentistry: A Literature Review

Amol Patil[1], Madhuri Patil[2], Inshiya Alazhar Contractor[3]

[1]Associate Professor, M.A Rangoonwala College of Dental Sciences
[2,3]Pediatric and Preventive Dentist, Private Practitioner

Abstract:
Dental caries is a problem that affects people's quality of life on a global scale, however secondary caries still accounts for the majority of reported failures. Based on its commercial and scientific potential, nanotechnology is recognized as an emergent 21st-century technology. It is expanding quickly and has numerous potential uses in dentistry. Newer silver compounds, such as Nano Silver Fluoride (NSF), have been discovered as a result of the demand for newer materials with an aesthetic focus and the establishment of a healthy balance between pathogenic and protective aspects. NSF is directly applied to the carious lesions to arrest and treat dental caries. It can be advantageous to treat dental caries in children since the management of dental caries with NSF is noninvasive and very comfortable to perform. This review offers an in-depth exploration of the progression, functioning, and practical implications of NSF in the prevention of dental caries.

Keywords: Caries prevention, Nano-silver fluoride, Nanotechnology, Primary teeth, Silver diamine fluoride

1. INTRODUCTION

In the last two decades, there has been a significant shift in the dentistry field; however, dental caries remains a prevalent chronic ailment globally. With oral healthcare demands surpassing the capacity of healthcare organizations, a considerable number of cavities remain untreated (1). Contemporary approaches to caries management involve non-invasive techniques such as caries arresting agents, silver compounds, sealants, resin infiltration, fluoride varnishes, toothpaste, and gels (2).

One notable compound, Silver diamine fluoride (SDF), has emerged as a highly effective means to halt caries progression. It is an alkaline, transparent solution comprising diamine-silver and fluoride ions. SDF stands out as the sole ion-based topical fluoride material that combines the remineralization of dental structures, akin to sodium fluoride, with the antibacterial impact on caries microorganisms through silver nitrate's action (3). Various studies (4,5) have demonstrated its efficacy against cariogenic bacteria and fungi, as well as its potential for remineralization on both enamel and dentin (6,7). However, a significant drawback limiting its widespread acceptance is the black discoloration SDF causes on carious lesions due to silver particle precipitation on the affected tissue (8). Other drawbacks of SDF encompass a metallic taste, transient skin staining lasting 2 to 14 days, and minor painful lesions if the SDF solution comes into accidental contact with oral mucosa, typically resolving within a couple of days.

To address the issue of undesired staining, a proposed strategy involves a combined approach wherein potassium iodide (KI) is applied immediately after the use of Silver diamine fluoride (SDF) (9), or a mixture of SDF with the glutathione (GSH) biomolecule is used (10). This combined protocol has demonstrated a positive outcome in minimizing staining compared to the singular application of SDF (10). However, the use of KI has been associated with reduced efficacy in caries control (11), and some level of staining affecting aesthetics has been observed in arrested carious lesions.

The progression in nanotechnology has led to the creation of silver nanoparticles (AgNPs), whose antibacterial properties are well-established in the medical field. AgNPs have garnered attention in dental research across various disciplines, including endodontics, restorative dentistry, orthodontics, implantology, prosthodontics, and periodontics, primarily for disinfection, prophylaxis, and the prevention of oral infections due to their favorable antimicrobial characteristics (12).

Nano silver fluoride (NSF), an innovative experimental formulation based on nanotechnology, integrates remineralizing and antimicrobial properties through silver nanoparticles, chitosan, and fluoride. It was designed to be an effective anti-caries agent without causing black staining on carious dental tissues (13). This lack of discoloration is attributed to the size of silver particles and the fact that nanoparticles do not undergo oxidation (14). This novel formulation is deemed safe for human use, with controlled clinical trials affirming its anti-caries efficacy. Nevertheless, the current scientific literature lacks a comprehensive review of the benefits associated with utilizing NSF in the treatment of dental caries. The following literature review aims to provide an understanding of the clinical significance and applications of NSF in dentistry.

2. Historical background:

China in 659 A.D	Use of silver in dentistry
G.V. Black in 1900s	Use of silver nitrate to stop carious lesions
Stebbins in 1981	Introduced silver nitrate, a combination of nitric acid and silver amalgam, to stop caries
Szabo in 1902	Concluded that penetration of silver nitrate through the whole carious lesion into the sound dentin was not more than 0.5 mm.
Howe	Used silver nitrate solution for the sterilization of the disintegrated dentin overlying the pulps of large carious lesions of first molars. He reduced the silver from the ammoniated solution with formalin.
Central Pharmaceutical Council of the Japanese Ministry of Health and Welfare in 1970.	First SDF product sold as Saforide
Targino et al	NSF was introduced

3. NANO-SILVER FLUORIDE

This novel experimental formulation comprises fluoride, chitosan, and silver nanoparticles (AgNPs). Similar to Silver Diamine Fluoride (SDF), it combines antibacterial properties with preventive qualities. Developed as a potent anticaries agent, special attention has been given to aesthetic considerations to counteract the discoloration associated with SDF. The resulting Nano Silver Fluoride (NSF) is a stable yellow-colored solution, demonstrating stability over a three-year period.

3.1 METHOD OF PREPARATION-

1.0g of chitosan undergoes dissolution in 200 ml of 2% acetic acid (V/V) to create colloidal silver. The solution is agitated overnight and subsequently subjected to vacuum filtration. Thirty minutes before the addition of sodium borohydride, a 60 ml aliquot of the chitosan solution is placed in an ice bath while being stirred, and 4.0 ml is introduced to a solution of silver nitrate at a concentration of X 0.012 mol L-1. The $AgNO_3$ to $NaBH_4$ ratio is maintained at 1:6 mass, and the addition is performed dropwise (15).
The reaction between $AgNO_3$ and $NaBH_4$ initiates almost immediately, causing the solution to transition from colorless to light yellow and eventually to reddish. The resultant silver nanoparticles exhibit a spherical shape with an average size of 3.2-1.2 nm. Sodium fluoride (NaF) is subsequently introduced to enhance the solution's stability. The concentrations of each component in micrograms per milliliter are as follows: **Chitosan[28,585 µg/ml]; Silver Ag^+ [376.5 µg/ml]; Sodium fluoride [5028.3 µg/ml]**.

4 MECHANISM OF ACTION

4.1 Antimicrobial action:

Streptococcus mutans (SM) is recognized as the primary cariogenic bacteria, playing a crucial role in the initiation and progression of carious lesions. Numerous studies highlight Nano Silver Fluoride (NSF) as an outstanding oral antibacterial agent, exhibiting effectiveness against cariogenic pathogens, particularly Streptococcus mutans (SM), and demonstrating inhibition of oral biofilm formation (16,17). The antibacterial properties of NSF are attributed to its silver nanoparticles (AgNPs), which possess optimal antibacterial efficacy influenced by factors such as concentration, type, and form.
Silver ions, a key component of AgNPs, exert their antibacterial effects by interacting with various structures within bacterial cells. These ions adhere primarily to the cell wall and cytoplasmic membrane through electrostatic attraction and an affinity for sulfur proteins, thereby enhancing membrane permeability and disrupting these crucial structures. The impact of AgNPs extends to damaging bacterial DNA, proteins, and lipids. Additionally, AgNPs induce oxidative stress responses, leading to bacterial cell destruction and an increase in the dephosphorylation of tyrosine residues on bacterial peptide substrates, ultimately inhibiting bacterial growth and viability (18). Remarkably, the antibacterial activity of silver nanoparticles against Streptococcus mutans is reported to be 25 times stronger than chlorhexidine, particularly when the nanoparticles have diameters between 80 and 100 nm, although cytotoxicity may rise with dimensions smaller than 20 nm.
The antimicrobial effectiveness of nano silver demonstrates an inverse relationship with its nanoparticle size, with smaller sizes exhibiting stronger efficacy. Studies (19,20) confirm that the antibacterial activity of AgNPs increases as their particle size decreases. Notably, investigations into NSF formulations reveal the presence of AgNPs with dimensions of 2.56 ± 0.43 nm, 3.2 ± 1.2 nm, and 5.9 ± 3.8 nm, contributing to enhanced antibacterial activity against Streptococcus mutans (21).
A study by PLL Freire et al. (2014) suggests that smaller AgNPs exhibit greater antibacterial activity due to their increased contact area with the bacterial cell surface. Furthermore, research indicates that AgNPs of different sizes and morphologies, including spheres, triangles, and ellipses, demonstrate the ability to prevent Streptococcus mutans biofilms (22).
Chitosan plays a crucial role as a stabilizing ingredient in NSF formulation. It converts silver ions to neutral ions, thereby stabilizing the particles by forming a thin cladding layer on their surface (23). Chitosan, known for its effectiveness against a wide range of Gram-positive or negative bacteria, adds to the overall antibacterial prowess of the NSF formulation.

4.2 Prevention of Biofilm Formation

Silver nanoparticles (AgNPs) exhibit biocidal and anti-adhesive microbial properties, effectively limiting the growth of biofilm-forming bacteria and preventing biofilm formation. Studies, such as the one conducted by Freire et al. in 2015, highlight that AgNP colloids prevent the growth of Streptococcus mutans (SM) biofilm on the surfaces of bovine enamel (24).

In a study by Sri Angky Soekanto et al., the effectiveness of Nano Silver Fluoride (NSF) and propolis fluoride (PPF) was investigated concerning biofilm development in Streptococcus mutans (SM) and Enterococcus faecalis (EF). The findings indicated that both NSF and PPF demonstrated a dose-dependent reduction in biofilm formation (25).

4.3 Prevent demineralization:

Silver nanoparticles (AgNPs) play a multifaceted role in preventing caries by addressing various aspects of enamel health. They are shown to reduce the production of lactic acid in biofilm, which is crucial in minimizing the demineralizing effect caused by acids produced in the biofilm. Studies indicate that sound enamel treated with AgNPs experiences a reduced mineral loss after biofilm challenge, demonstrating the potential of AgNPs to prevent caries by mitigating the demineralization process.

In a chemical model devoid of bacteria, AgNPs were found to increase the microhardness of enamel caries. AgNPs can penetrate carious lesions and attach to hydroxyapatite crystals. Moreover, silver ions released from AgNPs can form insoluble silver chloride on dental hard tissue, contributing to increased mineral density. The precipitated AgNPs and insoluble silver chloride not only enhance the mineral content but also preserve exposed collagen in carious teeth. This preservation is significant as exposed collagen in the oral environment can be susceptible to degradation by bacterial collagenases and other proteinases. AgNPs have demonstrated the ability to inhibit and deactivate these enzymes, thereby preserving collagen. The preserved collagen, in turn, acts as a scaffold for the deposition of mineral crystals, preventing further diffusion of calcium and phosphate.

A study conducted by Scarpelli et al. in 2017 explored the remineralization effect of AgNPs. The findings revealed that, besides their well-documented antibacterial properties against Streptococcus mutans, Enterococcus faecalis, and Escherichia coli, AgNPs also played a role in preventing the demineralization of deciduous tooth enamel (26).

4.4 Remineralization of teeth

While silver nanoparticles (AgNPs) demonstrate positive effects on dental hard tissue, it is acknowledged that fluoride exerts a more profound remineralizing impact. Fluoride's interaction with the mineral component of teeth results in the formation of fluorohydroxyapatite (FHAP or FAP), achieved by replacing OH- with F-. This process enhances hydrogen bonding, creates a more rigid crystal lattice, and reduces overall solubility. In addition to its anti-demineralization properties, fluoride interacts with enamel to prevent disintegration through remineralization. Fluoride inhibits bacterial enzyme enolase, impeding the production of bacterial acids, and also hinders dentin collagen degradation by inhibiting collagenases. Therefore, researchers propose the use of Nano Silver Fluoride (NSF) to promote the remineralization of enamel and dentin.

A study by V. Silva et al. in 2019 investigated NSF's role in the enamel remineralization process using optical coherence tomography. The findings indicated that the presence of silver nanoparticles did not interfere with the action of fluoride, highlighting NSF's inherent ionic stability. This discovery holds significance for deciduous teeth due to their unique enamel features. The degradation trajectories for the NaF and NSF groups were comparable, leading to the conclusion that NSF is as effective as sodium fluoride in remineralizing tooth enamel (27).

In 2017, Ali Nozari et al. discovered that the NSF group exhibited the highest surface microhardness (SMH) readings and percentage of surface hardness recovery (SMHR), suggesting that NSF demonstrated superior remineralization efficiency (28).

Amitis Vieira Costa e Silva et al. in 2018 demonstrated the efficiency of both NSF and sodium fluoride in remineralizing enamel. However, NSF outperformed sodium fluoride in buffering the decrease in pH and preventing the adhesion of Streptococcus mutans (SM) to the enamel surface. The study concluded that NSF is more efficient than sodium fluoride in treating initial carious lesions due to its combined remineralization and antibacterial characteristics (29).

5. Evidence based studies on the efficiency of NSF:

Numerous studies have extensively explored the application of Nano Silver Fluoride (NSF) for caries arrest and prevention:

1. Santos et al. (2014) conducted a randomized control trial comparing NSF to water, revealing that by the seventh day, NSF hardened and prevented dentine cavities in primary teeth 81 percent more efficiently than a placebo (water). Moreover, 66.7 percent of caries arrest occurred by the 12th month (30).
2. Valdeci Elias's 2017 study found that NSF effectively halted active dentine caries without noticeable tissue darkening, a common issue related to the oxidation of silver ions upon contact with teeth (31).
3. Tirupathi, Sunnypriyathan, et al., in 2019 discovered that the Nano-Silver integrated Sodium Fluoride (NSSF) group exhibited a higher caries arrest rate (77%) in reducing dentine caries formation in primary teeth compared to silver diamine fluoride (32). The bactericidal ability of NSSF is particularly crucial in primary teeth, which have more permeable enamel and less coverage than permanent teeth, making them more susceptible to acidogenic bacterial activity leading to carious lesions.
4. Zuhair Al-Nerabieah et al. in 2020 found that a new nano-silver fluoride formulation, including green tea extract, effectively altered carious lesions in deciduous teeth, with a six-month complete arrest rate of 67.4%. Lesions treated with standard silver diamine fluoride (SDF) showed significantly more arrest than those treated with NSF-GTE, possibly due to the higher silver nitrate and sodium fluoride concentrations in SDF (33).
5. Nanda K J et al. in 2020 demonstrated the efficacy of NSF in secondary caries prevention. Pre-treated cavities filled with glass ionomer cement (GIC) and composite resin showed significantly greater mean microhardness values than untreated cavities, indicating the remineralizing effect of NSF. Pre-treatment also resulted in a decreased outer lesion depth, suggesting enhanced tooth restoration stability (34).
6. Zuhair Al-Nerabieah et al. in 2020 assessed the cariostatic efficacy of NSF and SDF in primary teeth of preschool children. After three weeks, 77 percent of arrested carious lesions were found in the NSF group, decreasing to 67.2% by six months, slightly lower than the SDF group. Both SDF and NSF effectively reversed carious dentine lesions in preschool children and were well tolerated (35).
7. Arnaud M et al. in 2021 established the effectiveness of two different silver nanoparticle (AgNP) concentrations, 400 and 600 ppm, in preventing caries. NSF 600 had a higher success rate of 72.7%

compared to NSF 400, indicating that increased AgNP concentration contributed to higher efficacy (36).

8. A systematic review in 2022, including 15 articles, concluded that nano silver fluoride varnish is an effective agent for controlling demineralization of primary teeth, potentially replacing silver diamine fluoride (37).
9. Another systematic review and meta-analysis in 2022 by Choubey Shikha et al. found that NSF is an effective means for caries arrest in primary teeth (38).

6. METHOD OF APPLICATION

According to Valdeci Elias dos Santos Jr et al. (2014), the recommended Nano Silver Fluoride (NSF) application technique is as follows:

1. **Avoid Excavation:** There is no necessity to excavate carious dentin or remove any supporting enamel prior to applying Nano Silver Fluoride. Excavation may lead to a reduction in the proportion of carious lesions.
2. **Cleansing the Lesion:** Begin by cleansing the carious lesion with a wet cotton pellet to eliminate any residual food particles.
3. **Tooth Isolation:** Isolate the tooth intended for NSF treatment using a cotton roll or gauge piece.
4. **Application with Micro Brush:** Utilize a sterile micro brush tip to apply two drops of the NSF solution (equivalent to 10mg) to the carious lesion for approximately ten seconds.
5. **Prevent Cross-Contamination:** Ensure the use of a single micro brush to prevent cross-contamination during the application.
6. **Optimal Exposure Time:** Allow the solution to remain on the tooth surface for about 2 minutes to achieve the best effect.

7. ADVANTAGES

1. **Caries Arrest in Primary Teeth:** Nano Silver Fluoride has demonstrated effectiveness in arresting carious lesions in primary teeth.
2. **Non-Invasive Treatment:** It provides a non-invasive treatment option for arresting dental caries, eliminating the need for carious lesion excavations.
3. **No Staining:** Due to the incorporation of nano silver, which does not form silver oxide when exposed to air or oxygen, Nano Silver Fluoride does not stain the tooth. Unlike Silver Diamine Fluoride (SDF), where staining can occur due to the precipitation and oxidation of silver particles on carious dentin, NSF formulations avoid this issue (39). Studies, such as the one by Sayed et al., show no observed color change over time with the reduction in the size of silver nanoparticles (40). While Espíndola-Castro et al. noted yellowish stains on teeth two weeks after NSF application, these stains were removable by toothbrushing or gauze, and the tooth color was restored to baseline values (41).
4. **Remineralization and Prevention of Secondary Caries:** NSF has the capability to remineralize and prevent the progression of secondary caries.
5. **Antimicrobial Properties:** It possesses antimicrobial properties, contributing to the prevention of dental biofilm formation on the tooth surface.
6. **Minimal Equipment Requirement:** The application of NSF on the tooth surface requires minimal armamentarium, making it a convenient option.
7. **Cost-Effective:** NSF is considered a cost-effective solution for dental treatment.

8. Safety:

Targino et al. (2014) conducted a study evaluating the cytotoxic activity of NSF compared to chlorhexidine and SDF. The findings revealed that NSF was not hazardous at any concentration tested for any type of erythrocyte. Additionally, NSF demonstrated bacteriostatic and bactericidal properties, with statistically significant differences in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values when compared to chlorhexidine and SDF (42).

In a study by Sayed et al. (40), SDF was observed to cause distortion in dentin collagen morphology, although it provided an intrafibrillar pattern of mineral deposition. In contrast, NSF preserved collagen structural morphology and facilitated intrafibrillar remineralization. The change in collagen fiber morphology was attributed to the high pH of SDF (pH 10-12) compared to NSF (pH 8-9), suggesting better biocompatibility with the use of NSF. This indicates that NSF can serve as an alternative agent to SDF.

According to Freire et al. (2015), nano-silver exhibits less cytotoxicity than other dental materials. Their study assessed the cytotoxicity of silver nanoparticles (AgNPs) colloids on dental enamel, revealing that these colloids had a lower silver concentration, were less toxic, and had a bactericidal impact on bacteria associated with dental caries (22). This further supports the biocompatibility of nano-silver, making NSF a promising option for dental applications.

9. SCOPE FOR IMPROVEMENT-

Randomized Controlled trials with large sample size and long term follow up should be carried out to know the effectiveness of the material on caries in permanent teeth, root caries, dentinal sensitivity.

10. CONCLUSION

Nano Silver Fluoride (NSF) emerges as a promising avenue for directly halting carious lesions, presenting itself as a superior alternative to Silver Diamine Fluoride (SDF) by addressing discoloration concerns. The non-invasive application of NSF not only ensures an aesthetically pleasing outcome but also avoids the drawbacks associated with SDF. Its harmless and cost-effective nature positions it as a viable option for both pediatric and adult populations, seamlessly integrating into dental health regimens. The remarkable advantages of NSF make it highly applicable for inclusion in public health initiatives. However, a more extensive investigation involving larger sample sizes is imperative to ascertain the enduring efficacy of this material over the long term.

11. REFERENCES

1. Zuhair Al-Nerabieah , Ettihad Abo Arrag, John C Comisi, Anas Rajab. Effectiveness of a Novel Nano-Silver Fluoride with Green Tea Extract Compared with Silver Diamine Fluoride: A Randomized, Controlled, Non-Inferiority Trial. Int J Dentistry Oral Sci. 2020;7(6):753-761.
2. Contractor IA, M.S. G, M.D. I. Silver Diamine Fluoride: Extending the spectrum of Preventive Dentistry, a literature review. Pediatr Dent J. 2021;31(1):17–24.
3. Ana Claudia chibinski, Leticia wambier et al. Silver diamine fluoride has efficacy in contolling caries progression in primary teeth: A systematic review and meta analysis. Caries research 2017; 51:527-541 .
4. Fakhruddin KS, Egusa H, Ngo HC, Panduwawala C, Pesee S, Venkatachalam T, et al. Silver diamine fluoride (SDF) used in childhood caries management has potent antifungal activity against oral

Candida species. BMC Microbiol 2020;20;95-105
5. Zhao IS, Gao SS, Hiraishi N, Burrow MF, Duangthip D, Mei ML, et al. Mechanisms of silver diamine fluoride on arresting caries: a literature review. Int Dent J 2018;68:67–76
6. Yu OY, Zhao IS, Mei ML, Lo ECM, Chu CH. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. J Dent 2018;78:65–71
7. Mei ML, Ito L, Cao Y, Li QL, Lo ECM, Chu CH. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. J Dent 2013;41:809–817
8. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro. Int J Paediatr Dent 2018;28;514-522
9. Zhao IS, Mei ML, Burrow MF, Lo EC-M, Chu C-H. Effect of silver diamine fluoride and potassium ıodide treatment on secondary caries prevention and tooth discolouration in cervical glass ıonomer cement restoration. Int J Mol Sci 2017;18:340-345
10. Sayed M, Matsui N, Hiraishi N, Nikaido T, Burrow MF, Tagami J. Effect of glutathione bio- molecule on tooth discoloration associated with silver diammine fluoride. Int J Mol Sci 2018;9:1322-1330
11. Turton B, Horn R, Durward C. Caries arrest and lesion appearance using two different silver fluoride therapies on primary teeth with and without potassium iodide: 12-month results. Clin Exp Dent Res 2020 Dec 02. Doi:10.1002/cre2.367
12. Yin IX, Zhao IS, Mei ML, Li Q, Yu OY, Chu CH. Use of silver nanomaterials for caries prevention: A concise review. Int J Nanomedicine 2020;15:3181–3191
13. Sayed M, Hiraishi N, Matin K, Abdou A, Burrow MF, Tagami J. Effect of silver-containing agents on the ultra-structural morphology of dentinal collagen. Dent Mater 2020;36:936–944
14. Espíndola-Castro LF, Rosenblatt A, Galembeck A, Monteiro G. Dentin staining caused by nano-silver fluoride: A comparative study. Oper Dent 2020;45:435–441
15. dos Santos VE, Filho AV, Ribeiro Targino AG, Pelagio Flores MA, Galembeck A, Caldas AF, et al. A New "Silver-Bullet" to treat caries in children – Nano Silver Fluoride: A randomized clinical trial. Journal of Dentistry, 2014; 42(8): 945-51.
16. Vieira Costa e Silva, A., Teixeira, J. A., Mota, C. C. B. O., Clayton Cabral Correia Lins, E., Correia de Melo Júnior, P., de Souza Lima, M. G., et al. (2018). *In Vitro* morphological, Optical and Microbiological Evaluation of Nanosilver Fluoride in the Remineralization of Deciduous Teeth Enamel. *Nanotechnol. Rev.* 7 (6), 509–520. doi:10.1515/ntrev-2018-0083
17. Waikhom, N., Agarwal, N., Jabin, Z., and Anand, A. (2022). Antimicrobial Effectiveness of Nano Silver Fluoride Varnish in Reducing Streptococcus Mutans in Saliva and Plaque Biofilm when Compared with Chlorhexidine and Sodium Fluoride Varnishes. *J. Clin. Exp. Dent.* 14 (4), e321–e328. doi:10.4317/jced.59093
18. Cheng L, Li R, Liu G, et al. Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *Int J Nanomed*. 2018;**13**:3311–3327. doi: 10.2147/IJN.S165125
19. Baker C, Pradhan A, Pakstis L, Pochan DJ, Shah SI. Synthesis and antibacterial properties of silver nanoparticles. J Nanosci Nanotechnol 2005;5:244–249
20. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology 2005;16:2346–2353
21. Santos VE dos Jr, Vasconcelos Filho A, Targino AGR, Flores MAP, Galembeck A, Caldas AF Jr, et -al. A new “silver-bullet” to treat caries in children--nano silver fluoride: a randomised clinical trial. J

Dent 2014;42:945–951
22. Freire PL, Stamford TC, Albuquerque AJ, Sampaio FC, Cavalcante HM, Macedo RO, et al. action of silver nanoparticles towards biological systems: cytotoxicity evaluation using hen's egg test and inhibition of Streptococcus mutans biofilm formation. International journal of antimicrobial agents, 2015; 45(2): 183-7.
23. Hasan KMF, Wang H, Mahmud S, Jahid MA, Islam M, Jin W, et al. Colorful and antibacterial nylon fabric via in-situ biosynthesis of chitosan mediated nanosilver. Journal of Materials Research and Technology, 2020; 9(6): 16135-45.
24. Freire PL, Stamford TC, Albuquerque AJ, Sampaio FC, Cavalcante HM, Macedo RO, Galembeck A, Flores MA, Rosenblatt A. Action of silver nanoparticles towards biological systems: cytotoxicity evaluation using hen's egg test and inhibition of Streptococcus mutans biofilm formation. Int J Antimicrob Agents. 2015 Feb;45(2):183-7. doi: 10.1016/j.ijantimicag.2014.09.007.
25. Soekanto SA, Marpaung LJ, Djais AA, Rina RJIJoAP. Efficacy of propolis fluoride and nano silver fluoride for inhibition of streptococcus mutans and enterococcus faecalis biofilm formation. 2017;9(Special Issue 2):51-4.
26. Scarpelli, Beatriz & Punhagui, Marília & Hoeppner, M.G. & Almeida, Ricardo & Juliani, Felipe & Guiraldo, Ricardo & Berger, Sandrine. (2017). In Vitro Evaluation of the Remineralizing Potential and Antimicrobial Activity of a Cariostatic Agent with Silver Nanoparticles. Brazilian Dental Journal. 28. 738-743. 10.1590/0103-6440201701365.
27. Silva, A. V. C., Teixeira, J. D. A., Melo, P. C. D., Lima, M. G. D. S., Mota, C. C. B. D. O., Lins, E. C. C. C., et al. (2019). Remineralizing Potential of Nano-Silver-Fluoride for Tooth Enamel: an Optical Coherence Tomography Analysis. *Pesqui. Bras. em Odontopediatria Clínica Integr.* 19. doi:10.4034/pboci.2019.191.50
28. Nozari A, Ajami S, Rafiei A, Niazi E. Impact of Nano Hydroxyapatite, Nano Silver Fluoride and Sodium Fluoride Varnish on Primary Teeth Enamel Remineralization: An In Vitro Study. J Clin Diagn Res, 2017; 11(9): ZC97-ZC100.
29. Silva AVCe, Teixeira JA, Mota CCBO, Lins ECCC, Júnior PCdM, Lima MGdS, et al. In Vitro morphological, optical and microbiological evaluation of nanosilver fluoride in the remineralization of deciduous teeth enamel: %J Nanotechnology Reviews, 2018; 7(6): 509-20
30. dos Santos, V. E., Filho, A. V., Ribeiro Targino, A. G., Pelagio Flores, M. A., Galembeck, A., Caldas, A. F., et al. (2014). A New "Silver-Bullet" to Treat Caries in Children - Nano Silver Fluoride: A Randomised Clinical Trial. *J. Dent.* 42 (8), 945–951. doi:10.1016/j.jdent.2014.05.017
31. dos Santos Junior, V. E., Targino, A. G. R., Flores, M. A. P., Rodríguez-Díaz, J. M., Teixeira, J. A., Heimer, M. V., et al. (2017). Antimicrobial Activity of Silver Nanoparticle Colloids of Different Sizes and Shapes against Streptococcus Mutans. *Res. Chem. Intermed.* 43 (10), 5889–5899. doi:10.1007/s11164-017-2969-5
32. Tirupathi S, Svsg N, Rajasekhar S, Nuvvula S. Comparative cariostatic efficacy of a novel Nanosilver fluoride varnish with 38% silver diamine fluoride varnish a double-blind randomized clinical trial. J Clin Exp Dent, 2019; 11(2): e105-e12.
33. Al-Nerabieah Z, Arrag EA, Comisi JC, Rajab AJIJDOS. Effectiveness of a Novel Nano-Silver Fluoride with Green Tea Extract Compared with Silver Diamine Fluoride: A Randomized, Controlled, Non-Inferiority Trial, 2020; 7(6): 753- 61
34. Nanda, K. J., and Naik, S. (2020). An *In-Vitro* Comparative Evaluation of Pre-treatment with Nano-

Silver Fluoride on Inhibiting Secondary Caries at Tooth Restoration Interface. *Cureus* 12 (5), e7934. doi:10.7759/cureus.7934

35. Al-Nerabieah Z, Arrag E, Rajab AJJoS. Cariostatic efficacy and children acceptance of nano-silver fluoride versus silver diamine fluoride: a randomized controlled clinical trial, 73(3): 100-6.
36. Arnaud M, Junior PC, Lima MG, E Silva AV, Araujo JT, Gallembeck A, et al. Nano-silver Fluoride at Higher Concentration for Caries Arrest in Primary Molars: A Randomized Controlled Trial. International journal of clinical pediatric dentistry, 2021; 14(2): 207-11.
37. Aldubayyan, Abdulwahab & Alsuawari, Ahmed & Alotaibi, Bassam & Almalki, Hussam & Alkhamis, Faisal & Hussein, Bader & Ansari, Dr. (2022). Effect of Nanosilver Fluoride Varnish on Demineralized Primary Teeth: A Systematic Review. Saudi Journal of Oral and Dental Research. 7. 18-24. 10.36348/sjodr.2022.v07i01.004.
38. Choubey, Shikha; Patil, Amol; Talekar, Abhinav L.; Kalra, Dheeraj[1]. Nanosilver fluoride as a caries arresting agent in children: A systematic review and meta- analysis. Journal of Indian Society of Pedodontics and Preventive Dentistry 40(3):p 230-238, Jul–Sep 2022. | DOI: 10.4103/jisppd.jisppd_224_22
39. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro. Int J Paediatr Dent 2018;28;514-522
40. Sayed M, Hiraishi N, Matin K, Abdou A, Burrow MF, Tagami J. Effect of silver-containing agents on the ultra-structural morphology of dentinal collagen. Dent Mater 2020;36:936–944
41. Espíndola-Castro Galembeck A, Monteiro G. Dentin staining caused by nano-silver fluoride: A comparative study. Oper Dent 2020;45:435–441
42. Targino AG, Flores MA, dos Santos Junior VE, de Godoy Bené Bezerra F, de Luna Freire H, Galembeck A, et al. An innovative approach to treating dental decay in children. A new anti-caries agent. Journal of materials science Materials in medicine, 2014; 25(8): 2041-7.