ORIGINAL RESEARCH

Comparative Evaluation of the Mechanical Efficiency of Nanosilver Fluoride and Sodium Fluoride Varnish: An *In Vitro* Study

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Abstract

Background: Dental caries is the most common disease in childhood and has an impact on general health status. The topical application of fluoride varnishes has been used for the prevention and control of dental caries due to their high fluoride content, adhesion capacity, and safety. Silver has a varied application in medicine as well as in dentistry due to its anticaries, antimicrobial, and antirheumatic potentials. The introduction of nanosilver fluoride (NSF) was made with advancements in technology to overcome the drawbacks of silver diamine fluoride (SDF).

Aim: To compare and evaluate the microhardness and microleakage of NSF varnish and sodium fluoride (NaF) varnish.

Materials and methods: An *in vitro* comparative experimental study was carried out between synthesized NSF and commercially available NaF, with 20 samples in each group. The specimens were sectioned and subjected to microhardness evaluation using Vickers microhardness testing and the dye penetration method to evaluate the microleakage.

Results: The average microhardness was found to be 230.7218 VMH for NSF (group I), 198.9841 VMH for NaF (group II), and 91.6120 VMH for group III. These differences were statistically significant when compared with each other ($p = 0.002$). In 50% of the samples, no dye penetration was seen in the NSF group, compared to the NaF varnish group, where 75% of the samples exhibited penetration onto the varnish interface or the varnish and tooth interface.

Conclusion: Nanosilver fluoride proves to be an effective alternative to commercially available topical fluoride agents such as NaF. It has greater microhardness and lower microleakage than NaF and the control teeth.

Clinical significance: Nanosilver fluoride varnish can be used as a cost-effective alternative to NaF varnish and SDF. It can be applied with minimal training by healthcare workers or general practitioners.

Keywords: Microhardness, Microleakage, Nanosilver fluoride varnish, Sodium fluoride varnish, Topical fluoride.

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Introduction

Dental caries is a preventable disease when identified and treated early. It is most common in childhood and has an impact on general health status. Caries are caused by exposure to cariogenic biofilm and fermentable dietary carbohydrates. Chemical demineralization occurs due to microbial attack on dietary intake, causing a decrease in the pH of saliva and consequently demineralizing the dental enamel.[1,2] The initial demineralization of the enamel is a reversible process, as the hydroxyapatite crystals can regrow to their original sizes when exposed to a favorable remineralizing oral environment, such as an increase in salivary pH beyond the critical level.[3] Therefore, the topical application of fluoride varnishes has been used for the prevention and control of dental caries due to their high fluoride content, adhesion capacity, and safety. Over time, various fluoride-containing agents have been established, such as sodium fluoride (NaF), silver diamine fluoride (SDF), nanosilver fluoride (NSF), and so on.

These products are indicated according to an individual's exposure to dental caries. NaF varnish is used to prevent caries development, arrest early enamel, and even soft dentine caries by promoting remineralization of carious tooth substance.[4]

Silver has varied applications in medicine as well as dentistry due to its anticaries, antimicrobial, and antirheumatic potentials. SDF has the efficiency to prevent and halt coronal caries in primary teeth and root caries in permanent teeth due to its effects on *Streptococcus mutans* and lactobacilli.[5,6] Both silver and fluoride ions have been shown to inhibit cariogenic biofilms. They act directly against bacterial membranes by denaturing proteins and inhibiting DNA replication. Despite its efficacy, SDF has the disadvantage of causing black staining of tissues and a metallic taste. Therefore, the introduction of NSF was made with advancements in technology. The material's safety has been approved, and it does not stain dental structures like SDF. The formulation of nanosilver particles

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requires a biocompatible and stable carrier medium. Hence, in the search for such mediums, chitosan was identified.[5] Chitosan acts as a stabilizing agent and a reducing agent when used for the preparation of nanosilver particles, as it coordinates with the metal ions before reduction.[6] Hence, considering the environmental impact, nanosilver-containing materials should ideally be incorporated within biological structures while exhibiting their antimicrobial activity.

Hence, the aim and objective of the study were to compare and evaluate the microhardness and microleakage of NSF varnish and NaF varnish.

Materials and Methods

The present *in vitro* comparative experimental study was conducted at the Department of Pediatric and Preventive Dentistry, Pediatric and Preventive Dentistry, Maharishi Markandeshwar College of Dental Sciences and Research, Maharishi Markandeshwar (Deemed to be University), Ambala, Haryana, India, after obtaining institutional ethics clearance *via* IEC-1806. A total of 54 caries-free premolars with intact apex extracted due to orthodontic or periodontal reasons were included, while teeth with cracked teeth, root resorption, calcification, fracture, or root caries were excluded from the study. Around 22 teeth each were allocated to group I (NSF varnish) and group II (NaF varnish) for microhardness evaluation, with 10 teeth in group III (control group). For microleakage evaluation, 50 premolars meeting similar inclusion and exclusion criteria were included, with 20 teeth each allocated to group I (NSF varnish) and group II (NaF varnish) and 10 teeth in group III (control group).

Preparation of Nanosilver Particles

A 2% acetic acid solution (volume/volume) was prepared by mixing 4 mL of glacial acetic acid with 196 mL of distilled water. To this 2% acetic acid solution, 1 gm of chitosan was added to form a homogeneous mixture. The solution was stirred overnight using a magnetic stirrer at 1000 rpm, centrifuged at 400 rpm for 10 minutes, and filtered with Whatman No. 1 filter paper. Subsequently, a 60 mL aliquot of the chitosan solution was obtained. A solution of 10 mL of 0.012 mol L-1 silver nitrate was prepared by dissolving 20.4 mg of silver nitrate particles in 10 mL of distilled water. Around 4 mm of this silver nitrate solution was then added to the 60 mL of chitosan solution to prepare colloidal silver. To 122.4 mg of sodium borohydride, 8 mL of distilled water was added to maintain the same mass as that of silver nitrate. After being placed in an ice bath and stirred at 830 rpm for 30 minutes, 4 mL of this sodium borohydride solution was added. The ratio between $NaBH_4$ and silver nitrate ($AgNO_3$) was maintained at 6:1 by mass and added dropwise. The reduction of Ag+ ions was initiated immediately after the addition of sodium borohydride (at a 1:6 ratio by mass), causing the solution to change from colorless to light yellow to reddish-brown. In the end, after the solution stabilized in a color change to brownish-red, 300 mg of NaF was added.

This solution was further subjected to characterization using scanning electron microscopy (SEM) analysis to determine the size of the nanosilver particles. Fourier transform infrared spectroscopy (FTIR) analysis was conducted to detect the presence of silver particles in the synthesized solution.

Preparation of Demineralizing Solution

To prepare the demineralizing solution, 50 mM acetic acid, 2.2 mM sodium dihydrogen phosphate (NaH_2PO_4), and 2.2 mM calcium chloride ($CaCl_2$) were mixed with 200 mL of distilled water and adjusted to a pH of 4.8.

Preparation of Remineralizing Solution

The remineralizing solution contained 0.15 M potassium chloride (KCl), 1.5 mM $CaCl_2$, and 0.9 mM NaH_2PO_4 in 200 mL of distilled water, adjusted to a pH of 7.0.

Preparation of Specimen

For Microleakage Evaluation

Nanosilver fluoride varnish and NaF varnish were applied to a 3 × 3 mm enamel surface area of extracted premolars, respectively. The samples were then immersed in water for 10 days. The surface of the teeth was painted with two coats of nail varnish, leaving the test area unpainted. The apices of the teeth were sealed with sticky wax and then immersed in Basic Fuchsin solution for 24 hours. After rinsing, drying, and embedding in epoxy resin, each tooth was sectioned buccolingually through the center of the varnish application using a low-speed water-cooled diamond disk and straight handpiece and examined under a stereomicroscope.

For Microhardness Testing

Nanosilver fluoride varnish and NaF varnish were applied to a 3 × 3 mm enamel surface area of the extracted premolars. Both groups of teeth were subjected to artificial caries challenge for 14 days at room temperature by immersing them in demineralizing solution for 8 hours followed by remineralizing solution for 16 hours each day. Each tooth was then sectioned mesiodistally using a low-speed water-cooled diamond disk and straight handpiece. The sectioned teeth were mounted on epoxy resin and subjected to Knoop microhardness testing to determine the Vickers hardness values, applying a load of 980.7 mN for 10 seconds.

Scoring Criteria

The scoring criteria used for the dye penetration method was first introduced by Staninec and Holt,[7] in which they evaluated the marginal leakage of the restorative material. Butail et al.[8] adapted the dye penetration method to check the microleakage of fissure sealant on extracted teeth. Dye penetration was graded based on the extent of penetration along the surface of the varnish.

- Score 0: No dye penetration.
- Score 1: Dye penetration along the varnish interface.
- Score 2: Dye penetration at the tooth-varnish interface.
- Score 3: Dye penetration into the underlying tooth surface.

Results

The average microhardness was found to be 230.7218 VMH for NSF (group I), which was statistically significantly higher than NaF (group II), with 198.9841 VMH and 91.6120 VHM for the control group (group III) (Table 1). The microleakage scores were recorded for the dye penetration under a stereomicroscope at 2× magnification. The average score for group I was 0.6 and 1.1 for group II. The highest was seen in group III, at 2.6 (Table 2).

The experimental groups, when compared with the control group, showed a statistically significant difference in the microhardness values ($p = 0.020$), but the intergroup comparison between group I and group II was not found to be statistically significant ($p = 0.556$) (Tables 3 and 4).

There was no microleakage observed up to the tooth surface in the NSF varnish group, whereas the control group showed the

maximum microleakage into the tooth surface. In 50% of the samples, no dye penetration was seen in the NSF varnish group, compared to the NaF varnish group, where 75% of the samples exhibited penetration onto the varnish interface or the varnish and tooth interface. The difference between group I (NSF) and group II (NaF) at the varnish interface was only 5% higher in the NSF group. When comparing the level of the tooth interface, there was no penetration in the NSF group, whereas 35% of the NaF group and 40% of the control group showed penetration (Table 5 and Fig. 1).

Figure 2 depicts the FTIR spectra of the silver nanoparticles, chitosan, and silver nanoparticles loaded chitosan, with prominent peaks of silver nanoparticles observed at 1637 and 549 cm^{-1}.

The synthesized NSF varnish was subjected to SEM analysis to characterize the formed nanosilver particles. The particle sizes

Table 1: Average Vickers microhardness values for all the groups

Microhardness testing											
Weight: 0.98 N; time: 10 seconds											
NSF				*NaF*				*Control*			
Sample no.	*VMH*	*D1*	*D2*	*Sample no.*	*VMH*	*D1*	*D2*	*Sample no.*	*VMH*	*D1*	*D2*
22	230.722	30.8809	32.7482	22	198.984	32.3823	35.3441	10	91.612	62.61	61.805

Table 2: Average microleakage evaluation for all the groups using dye penetration

Microleakage								
NSF			*NaF*			*Control*		
n = 20	Average	0.6	*n* = 20	Average	1.1	*n* = 10	Average	2.6

Table 3: *Post hoc* statistical analysis for Vickers microhardness test for all three groups

					95% confidence interval	
Groups (I)		*Mean difference (I–J)*	*Standard error*	*Significance*	*Lower bound*	*Upper bound*
NaF	NSF	−31.73773	30.54950	0.556	−105.4836	42.0081
	Control	107.37209*	38.64240	0.020	14.0901	200.6541
NSF	NaF	31.73773	30.54950	0.556	−42.0081	105.4836
	Control	139.10982*	38.64240	0.002	45.8278	232.3918
Control	NaF	−107.37209*	38.64240	0.020	−200.6541	−14.0901
	NSF	−139.10982*	38.64240	0.002	−232.3918	−45.8278

*The mean difference is significant at the 0.05 level

Table 4: One-way statistical analysis for Vickers microhardness test for all three groups

					95% confidence interval for mean					
	N	*Mean*	*Standard deviation*	*Standard error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*	*F*	*Significance*
NaF	22	198.9841	101.55849	21.65234	153.9556	244.0126	80.49	438.60	6.567	0.003
NSF	22	230.7218	118.48772	25.26167	178.1873	283.2563	81.33	490.90		
Control	10	91.6120	36.73151	11.61552	65.3359	117.8881	70.19	166.50		
Total	54	192.0306	111.45712	15.16739	161.6086	222.4525	70.19	490.90		

Table 5: Statistical analysis for microleakage test for all three groups

			Microleakage scores				
			No dye penetration	*Dye penetration along varnish interface*	*Visible dye penetration at the dye and tooth interface*	*Complete dye penetration into tooth structure*	*Significance*
Groups	NaF	Count	5	8	7	0	0.000
		% within groups	25.0%	40.0%	35.0%	0.0%	
	NSF	Count	10	9	0	1	
		% within groups	50.0%	45.0%	0.0%	5.0%	
	Control	Count	0	0	4	6	
		% within groups	0.0%	0.0%	40.0%	60.0%	

ranged from 9.065 to 32.74 µm, with an average size of 19.48 µm (Fig. 3).

Ultraviolet-visible (UV-Vis) spectra revealed peaking of nanosilver particles at 400 nm. This was used to track nanosilver particle formation in the matrix of chitosan and confirm its presence in the material thus formed (Fig. 4). It was tested against $AgNO_3$ to differentiate the peaking of the particles.

Discussion

The growing need for more professionals to provide quality dental care in developing countries, along with a treatment regime suitable for general practitioners, has driven the introduction of newer materials with the potential to deliver high standards of physical, chemical, and mechanical properties, as well as biocompatibility.

One such new area is the development of "nanomaterials." Silver nanoparticles, in particular, have gained increased attention and prominence due to their well-established characteristics and properties.[9] The nanosilver particles are synthesized from silver nitrate using biopolymers such as chitosan as a biocompatible medium onto which the silver nanoparticles are integrated. This biostable medium plays a vital role in reducing the toxicity of the nanoparticles without inhibiting the antimicrobial activity, providing a synergistic effect on the biofilm when exposed.[10,11]

Sodium fluoride was chosen to be compared with the synthesized NSF due to its established properties and varied clinical applications. NaF, commonly used for topical fluoride therapy, facilitated the comparison of NSF properties within the same category. A positive control was included to eliminate errors and provide standardized results.

Characterization of the formed nanoparticles was conducted to determine their properties using FTIR analysis, SEM analysis, and UV-vis spectroscopy. FTIR spectra of the silver nanoparticles exhibited prominent peaks at 1637 and 549 cm^{-1} (Fig. 2) that were similar to those reported by Ibrahim et al. in 2018, who used chitosan preparation as antioxidant nanoparticles for the drug delivery system to enhance the anticancer drug.[12] The band at 1631 cm^{-1} in the spectra corresponds to C–N and C–C

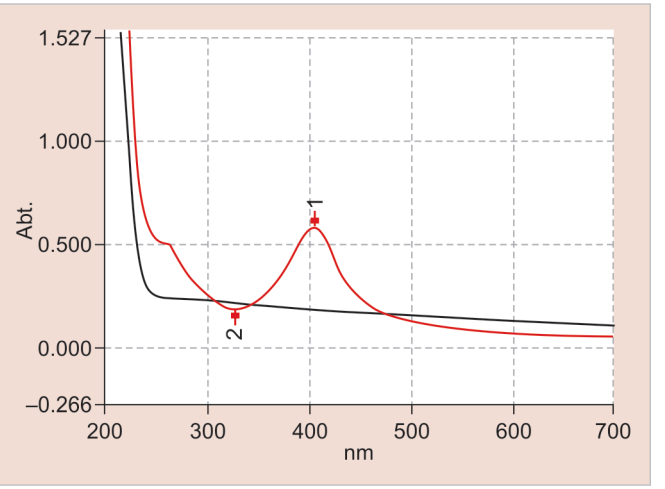

Fig. 2: Ultraviolet spectroscopy—determination of silver particle presence

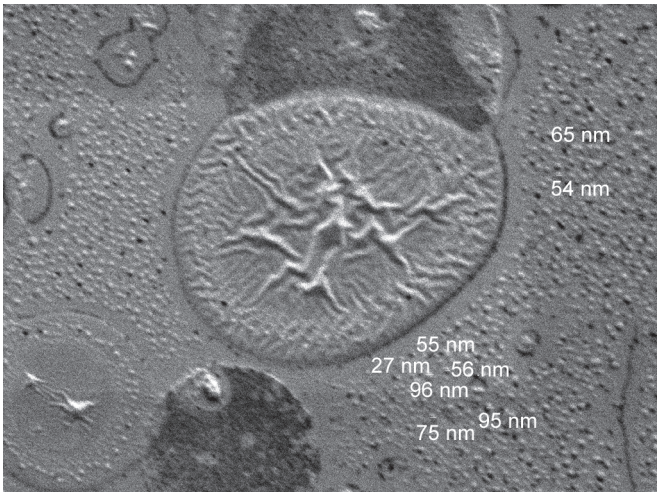

Fig. 3: Scanning electron microscopy analysis

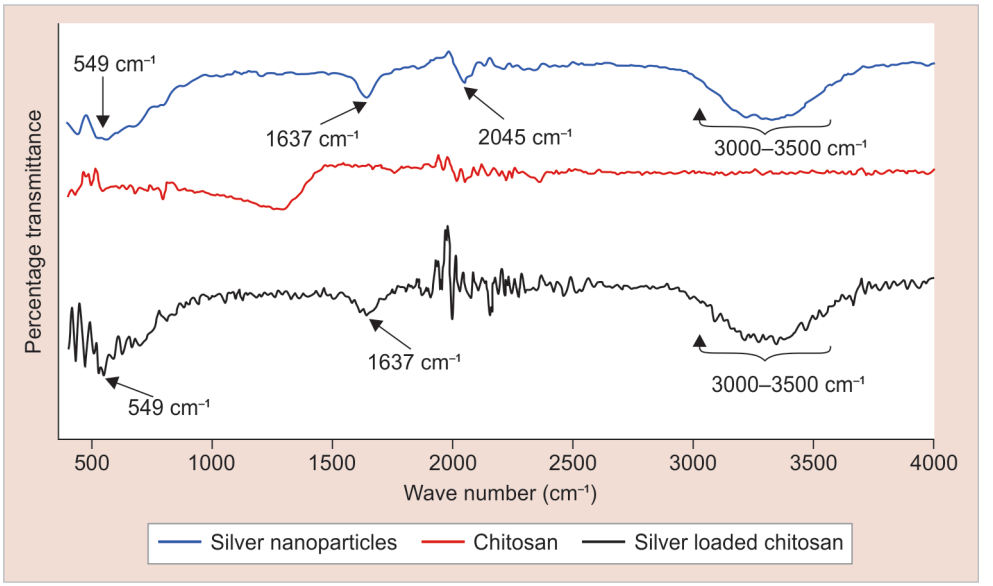

Fig. 1: Fourier transform infrared spectroscopy analysis of silver nanoparticles

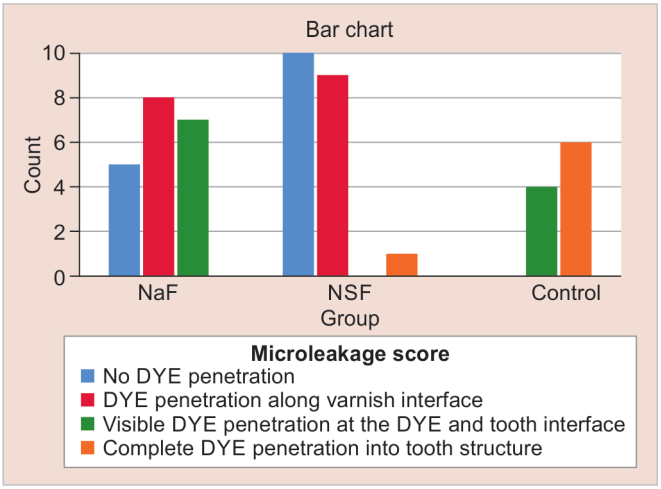

Fig. 4: Microleakage for all the three groups

stretching, indicating the presence of proteins that were similar to the findings of Prakash et al. in 2013.[13] The prominent peaks of silver nanoparticles are also presented in the FTIR spectrum of silver nanoparticles loaded chitosan, confirming that silver nanoparticles were successfully loaded onto chitosan in our study.

Scanning electron microscopy analysis of the formed nanosilver particles revealed particle sizes ranging from 9.065 to 32.74 µm, with an average size of 19.48 µm (Fig. 3). Targino et al. produced silver nanoparticles with a size of 5.9 ± 3.8 nm, which facilitated antimicrobial activity against *S. mutans*. Although various other studies have demonstrated sizes ranging from 5 to 98 nm.[14]

The NSF group had a mean microhardness of 230.7218, and the NaF group had 198.9841, whereas the control group had 91.6120 (Tables 1 and 4). This result was similar to that of Nozari et al., who also demonstrated a higher surface microhardness value of the NSF group compared to nanohydroxyapatite and NaF but found no statistically significant difference between the groups.[15] The NSF group exhibited a granular, heterogeneous protective layer mainly composed of calcium fluoride (CaF_2), which was not visible in the other groups. The diversity in crystalline lattice shape and size was mainly due to their nanoionic origins. Akyildiz and Sönmez reported higher microhardness in the NaF group compared to the NSF group, which contradicts the findings of the present study.[16]

A statistically significant difference in microhardness values was observed in the present study among the NSF, NaF, and control groups ($p = 0.002$) (Table 3), which contradicts a study by Teixeira et al. in 2018 that showed no statistical difference between the NSF group and NaF group in their microhardness values.[17]

Chitosan acts as both a stabilizing agent and a reducing agent in the preparation of nanosilver particles, as it coordinates with the metal ions before reduction. These reduced ions are further incorporated into the polymer chain and coupled with the oxidation of chitosan hydroxyl groups.[18] This will thereby form a strong chemical bond between the amino groups of the polymer and the silver ion particles. The smaller sizes of the nanoparticles are suggested to be potentially toxic without a biological agent attached to them, as their release into the surroundings might cause cytotoxic effects.[6] Hence, considering the environmental impact, nanosilver-containing materials should ideally be incorporated within biological structures while still exhibiting their antimicrobial activity.[11]

The dye penetration method was used to determine the microleakage of the materials, which was observed under a stereomicroscope at 2× magnification. The average microleakage value of the NSF group was lower (0.6) compared to that of the NaF (1.1) and control (2.6) groups (Table 2). The lower values of microleakage in the NSF group are attributed to the particulate nature of the silver ions, which embed onto the enamel surface and form a protective layer, whereas no such layer is formed in the NaF group. Shabzendedar et al. in 2011 showed higher microleakage values in the NaF group compared to the experimental groups.[19]

This contrasts with a study by Suzuki et al. in 1974, which evaluated the effect of SDF on tooth enamel and found that after SDF application, fluoride could penetrate the enamel up to approximately 25 µm in-depth, with large amounts of silver particles deposited onto the enamel surface. Some silver ions were also observed to penetrate approximately 20 µm into the enamel.[20] Similarly, Morales et al. in 2014 concluded that the silver nanoparticles addition did not alter the microleakage.[21] Moosavi et al. in 2013 stated that the precipitation of CaF_2 on the surface of NaF-treated hypomineralized enamel may have a significant inhibitory effect on microleakage.[22]

The observed microleakage at the varnish-tooth interface was <5% in the NSF group, whereas it was 35% in the NaF group and 40% in the control group. The varnish-tooth surface interface is a critical evaluation point, as bacterial loads commonly enter through these unnoticed cracks, causing secondary carious lesions by microorganism penetration. These microscopic spaces form due to improper bond formation or adhesion of the material, thereby leading to bacterial leakage.

The NSF group showed 95% positivity in microleakage resistance, as the dye penetrated only up to the varnish interface. The varnish's crystalline lattice exhibited spacing due to the dispersion of nanosilver particles, which absorbed the stain but were strong enough to prevent penetration into the underlying structures. The protective layer formed by the NSF group, composed of chitosan and aggregated silver nanoparticles, possesses strong bond strength and a spread-out lattice, along with antibacterial properties, contributing to lower microleakage values. Regiel et al. in 2012 stated that chitosan prevents the coalescence of nanoparticles, thereby aiding in their increased solubility and reducing microleakage.[18]

When silver nanoparticles are combined with fluoride, they produce a profound remineralizing effect on dental hard tissues. Fluoride combines with calcium ions, hydroxyl ions, and phosphate ions present in the dental matrix to form fluorapatite and fluorhydroxyapatite crystals, which are more resistant to enzymatic acid attacks than hydroxyapatite crystals. Fluoride also inhibits collagenases, thereby preventing dentinal collagen degeneration. Combining these elements for clinical use has gained popularity, as evidenced by their application in the present study.

Thus, the protective layers provided by the NSF varnish helped increase the microhardness and reduce the microleakage of the enamel structure, which could offer promising clinical advantages in less privileged communities.

The limitation of the study was that the sample size was small, and it was an *in vitro* study. Further application should be made to *in vivo* models to break through material efficacy and clinical usage.

Conclusion

Nanosilver fluoride proves to be an effective alternative to commercially available topical fluoride agents like NaF. It demonstrates superior physical properties, particularly in microhardness, by forming crystalline lattices. NSF exhibits greater microhardness compared to NaF and control teeth. Additionally, NSF shows lower microleakage, indicating its superior antimicrobial effect by inhibiting dye penetration onto the tooth surface. The topical applicability of NSF proves to be superior among the compared groups in the present study.

Clinical Significance

Hence, NSF could serve as a successful and cost-effective alternative to SDF, suitable for application by minimally trained healthcare workers or general practitioners.

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