Original Article

Effect of Nano-silver and Fluoride Toothpaste on Microhardness and Surface Roughness of Enamel White Lesions

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KEY WORDS
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ABSTRACT

Background: Enamel white spot lesions represent the earliest clinically detectable stage of dental caries and may be reversed through appropriate remineralization therapies. While fluoride-containing toothpastes are widely used for enamel remineralization, limited evidence is available regarding the effects of nano-silver-containing toothpastes on enamel surface properties. This study aimed to compare the effects of nano-silver and fluoride toothpastes on the microhardness and surface roughness of enamel white spot lesions.

Purpose: This study aimed to compare the effects of nano-silver and fluoride on the microhardness and surface roughness of enamel white lesions.

Materials and Method: An *in vitro* experimental study was conducted on freshly extracted, healthy permanent teeth, which were sectioned using metal discs to produce enamel samples. A total of 24 enamel samples were evaluated for initial microhardness (the Vickers tester) and surface roughness (the Profilometer). Subsequently, the samples were exposed to an acidified hydroxyethyl cellulose system for 96 hours to induce enamel white lesions. The microhardness and surface roughness of the lesions were assessed again. The samples were then randomly divided into two groups: Group I was treated with fluoride toothpaste, while Group II was treated with nano-silver toothpaste. Each pair of groups was analyzed by using repeated measures ANOVA.

Results: There were significant differences between the three measurement stages (p V-alue <0.001) in each group. In the Test of Between-Subjects Effects, the changes across all stages were similar between the two groups (p= 0.701). In both groups, microhardness and surface roughness increased after demineralization but decreased post-treatment in both groups.

Conclusion: Within the limitations of this in vitro study, nano-silver toothpaste demonstrated a remineralization potential comparable to that of conventional fluoride toothpaste. Both interventions improved enamel microhardness and reduced surface roughness following demineralization, suggesting that nano-silver toothpaste may serve as a potential alternative for the management of enamel white spot lesions.

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Introduction

Dental caries is a common infectious disease affecting the hard tissues of the tooth. Although oral health improvement programs are widely implemented, maintaining optimal oral health continues to be a major challenge in dentistry. There is growing emphasis on using protective and preventive measures before oral health deteriorates. Similarly, using conservative methods to prevent the need for cavity preparation has become increasingly important [1]. The demineralization process is dynamic and, if identified early, can potentially be reversed. Caries progression occurs when demineralization episodes outweigh remineralization phases. The loss of minerals due to disease progression leads to observable changes on the tooth surface, starting from a subclinical stage (white spot lesions) to the eventual formation of cavities [2].

Enamel white lesions are the earliest signs of tooth demineralization. At this stage, shifting the balance toward remineralization can prevent the further spread of these lesions. Identifying an optimal protocol for remineralizing early enamel lesions is a critical step toward achieving preventive and minimally invasive dentistry [3]. In recent years, non-invasive treatments for early caries have significantly increased, owing to a deeper understanding of mineral loss mechanisms [2].

Recent studies have suggested that nano-silver may be effective in preventing the formation of dental biofilm. Silver nanoparticles, due to their electrostatic attarction, can penetrate bacterial cell walls, induce oxidative stress, and subsequently interfere with bacterial DNA transcription and cellular respiration. It has also been reported that the size of silver particles enhances their antimicrobial activity due to increased surface contact with bacterial cells, without causing tooth discoloration commonly associated with silver diamine fluoride [4].

Toothpaste, while used in conjunction with toothbrushing, represents one of the most widely used mechanical and chemical approaches for plaque control. Fluoride is a well-established agent effective in preventing caries and halting the progression of new carious lesions, functioning as a remineralizing agent [1]. Nano-silver poses no significant health risks to human cells. *Streptococcus mutans*, identified as the most cariogenic bacterium in the oral cavity, has shown susceptibility to the bactericidal effects of silver nanoparticles [5].

Several studies have primarily focused on the antimicrobial properties of silver-based materials and their effects on cariogenic bacteria [4–6]. More recently, silver-containing agents have also been reported to improve enamel surface characteristics, including microhardness and surface integrity [4-6]. However, evidence regarding the remineralizing effects of nano-silver-containing toothpaste remains limited, and direct comparisons with conventional fluoride toothpaste are scarce. Therefore, the present study aimed to compare the effects of nano-silver and fluoride toothpastes on the microhardness and surface roughness of enamel white spot lesions under laboratory conditions.

Materials and Method

This *in vitro* experimental study was conducted on freshly extracted human permanent molars free of caries, restorations, fractures, enamel hypoplasia, erosion, and severe wear. Following extraction, all specimens were cleaned to remove soft tissue remnants and surface debris using a scalpel blade and a low-speed rotary brush under running water. The teeth were subsequently disinfected in 0.1% thymol solution for 48 hours. Standardized enamel specimens measuring approximately 5×5×2.5mm were prepared from the buccal and lingual surfaces using a water-cooled metal disc. To obtain uniform test surfaces, all specimens were polished using 600-grit Sof-Lex discs (3M ESPE, St. Paul, MN, USA) according to previously described protocols [3].

Baseline surface roughness and microhardness measurements were obtained for all specimens. Surface roughness was assessed using a TR200 profilometer [1], while enamel microhardness was measured using a Vickers digital microhardness tester (Bareiss, USA) [3]. For each specimen, measurements were recorded at three different locations and the mean value was considered the representative value for statistical analysis. Efforts were made to ensure homogeneity of baseline microhardness and surface roughness among the specimens. Artificial enamel white spot lesions were induced by exposing the specimens to an acidified hydroxyethylcellulose demineralizing system (pH 4.5) for 96 hours [7]. Following demineralization, the specimens were rinsed with distilled water for one minute and stored in a buffer solution (pH 7.4) at room temperature [3]. Surface roughness and microhardness measurements were then repeated to confirm lesion formation.

The demineralized enamel specimens were randomly allocated into two groups (n= 12 per group). Group 1 was treated with a fluoride-containing toothpaste (Colgate Regular®, Colgate-Palmolive Company, China, manufactured under European license), containing 1450 ppm fluoride as the active remineralizing agent. Group 2 was treated with a nano-silver-containing toothpaste

(Coral Nano Silver Toothpaste®, Coral LLC, USA), containing silver nanoparticles as the active ingredient. Each specimen was brushed three times daily for two minutes using a standardized pea-sized amount of the assigned toothpaste. The brushing protocol was continued for 15 consecutive days. The brushing duration was selected based on established oral hygiene recommendations indicating that approximately two minutes of brushing is required for adequate plaque removal and fluoride delivery. After each brushing session, the specimens were rinsed with distilled water for 15 seconds. Throughout the experimental period, all samples was maintained in a 100% humid environment using isotonic saline solution without additional remineralizing agents.

At the end of the intervention period, surface roughness and microhardness measurements were repeated using the same instruments and procedures employed at baseline. Microhardness measurements were performed using a Vickers microhardness tester (Bareiss, USA) under a load of 100g for 10 seconds [3]. Surface roughness was evaluated using the profilometer [1]. Three measurements were obtained from each specimen for both outcomes, and the mean value was used for statistical analyses. Statistical analyses were performed using SPSS software (Version 18; IBM Corp., Armonk, NY, USA). Data were expressed as mean±standard deviation (SD). Changes in microhardness and surface roughness across the three assessment stages (baseline, post-demineralization, and post-treatment) were evaluated using repeated-measures analysis of variance (ANOVA). A *p* Value < 0.05 was considered statistically significant.

Results

Microhardness

The microhardness measurements obtained at baseline, after demineralization, and after remineralization are presented in Table 1 and Figure 1. In the fluoride toothpaste group, the mean microhardness was 401.25±57.65 kg/mm² at baseline, decreased to 165.66±61.48 kg/mm² after demineralization, and increased to 328.08±91.94 kg/mm² following remineralization. Similarly, in the nano-silver toothpaste group, the mean microhardness was 466.16±65.30kg/mm² at baseline, decreased to 142.66±26.86kg/mm² after demineralization, and increased to 269.75±45.72kg/mm² after remineralization (Table 1, Figure 1). Repeated-measures analysis demonstrated statistically significant differences in microhardness across the three assessment stages ($p< 0.001$). The overall pattern of microhardness changes did not differ significantly between the fluoride and nano-silver toothpaste groups (Group×Time interaction, $p = 0.689$) (Table 1).

Surface Roughness

The surface roughness measurements at baseline, after demineralization, and after remineralization are presented in Table 2 and Figure 2. In the fluoride toothpaste group, the mean surface roughness was 0.370±0.223µm at baseline, increased to 0.536±0.271µm after demineralization, and decreased to 0.290±0.072µm after remineralization. In the nano-silver toothpaste group, the corresponding values were 0.430±0.203µm, 0.545±0.128µm and 0.289±0.060µm, respectively (Table 2, Figure 2).

Statistically significant differences in surface roughness were observed across the three assessment stages ($p< 0.001$). No statistically significant difference was observed between the fluoride and nano-silver toothpaste groups regarding the overall pattern of surface roughness changes over time (Group×Time interaction, $p= 0.701$) (Table 2).

Discussion

The present in vitro study evaluated and compared the effects of nano-silver and fluoride-containing toothpastes on the microhardness and surface roughness of artificially demineralized enamel. The findings demonstrated that both toothpastes significantly improved enamel microhardness and reduced surface roughness following demineralization. However, no statistically significant differences were observed between the two groups regarding the overall pattern of changes in either outcome. Enamel microhardness is widely recognized as an indirect indicator of mineral content and has been extensively used to evaluate enamel demineralization

Table 1: Enamel microhardness values (kg/mm²) at baseline, after demineralization, and after remineralization in the study groups

MH	Baseline			After Demineralization			After Remineralization			*p* Value
Groups	Max	Min	Mean ± SD	Max	Min	Mean± SD	Max	Min	Mean±SD	
Group1	460	276	401.25±57.65	332	116	165.66±61.48	541	192	328.08±91.94	
Group2	583	336	466.16±65.3	190	98	142.66±26.86	377	209	269.75±45.72	$p< 0.001$
p Value	$p= 0.689$			$p= 0.689$			$p= 0.689$			

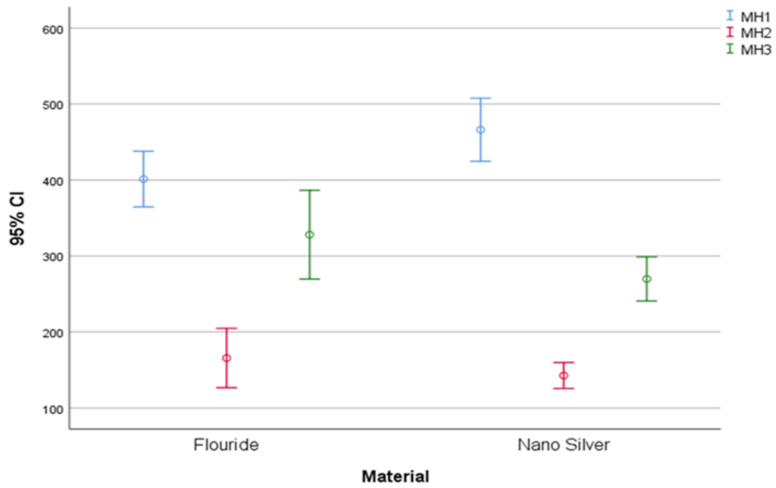

Figure 1: Changes in enamel microhardness (kg/mm²) across baseline, demineralization, and remineralization stages in the fluoride and nano-silver toothpaste groups

and remineralization processes [9-10,13]. In the present study, artificial demineralization resulted in a marked reduction in enamel microhardness in both groups, indicating successful lesion formation. Following the remineralization period, microhardness values increased in both the fluoride and nano-silver groups, suggesting that both interventions contributed to partial recovery of enamel mineral integrity.

The observed findings are consistent with previous studies investigating the remineralizing potential of nano-silver fluoride (NSF)-based materials. Zhi *et al.* [11] demonstrated that silver and fluoride ions could enhance the remineralization of demineralized enamel and dentin under laboratory conditions. Similarly, El-Desouky *et al.* [12] reported that NSF and fluoride varnish exhibited comparable anticariogenic effects and were equally effective in limiting enamel demineralization in primary teeth. Furthermore, Vieira Costa e Silva *et al*. [14] showed that NSF solution produced remineralization outcomes comparable to those achieved with sodium fluoride in primary enamel lesions.

The findings of the present study are also in agreement with those reported by Teixeira *et al*. [5], who evaluated a nano-silver fluoride-containing dentifrice and found that its ability to prevent enamel demineralization was comparable to that of conventional sodium fluoride toothpaste. Using microhardness testing and optical coherence tomography, the authors demonstrated that NSF-containing toothpaste effectively reduced mineral loss during pH cycling challenges. Since sodium fluoride toothpaste is considered a standard preventive approach against enamel demineralization, these findings support the potential application of nano-silver-containing formulations as alternative preventive agents. In contrast, Akyildiz and Sönmez [4] reported that NSF varnish produced lower remineralization efficacy than sodium fluoride varnish when evaluated using enamel microhardness measurements. Differences between their findings and those of the present study may be related to variations in study design, formulation type, fluoride concentration, application protocol, exposure time, and characteristics of the enamel substrate. Such methodological differences should be considered when comparing outcomes across studies.

In addition to microhardness, surface roughness represents an important parameter for evaluating enamel surface integrity. Increased roughness may facilitate bacterial adhesion and plaque accumulation, thereby in creasing susceptibility to caries progression [17]. In the present study, surface roughness increased following

Table 2: Mean ± SD, minimum, and maximum values of enamel surface roughness (Ra, µm) at baseline, after demineralization, and after remineralization in the fluoride and nano-silver toothpaste groups

Ra	Baseline			After Demineralization			After Remineralization			*p* Value
Groups	**Max**	**Min**	**Mean ±SD**	**Max**	**Min**	**Mean ±SD**	**Max**	**Min**	**Mean ±SD**	
Group1	0.83	0.05	0.370±0.223	1.11	0.16	0.536±0.271	0.44	0.19	0.290±0.072	
Group2	0.79	0.12	0.430±0.203	0.70	0.30	0.545±0.128	0.42	0.17	0.289±0.060	p< 0.001
p Value	*p*= 0.701			*p*= 0.701			*p*= 0.701			

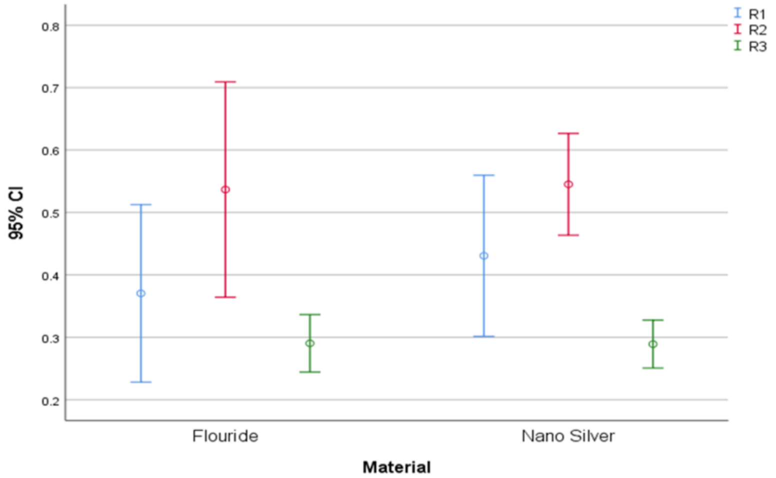

Figure 2: Changes in enamel surface roughness (Ra, µm) across baseline, demineralization, and remineralization stages in the fluoride and nano-silver toothpaste groups (95% confidence intervals)

demineralization and decreased after treatment in both experimental groups. Moreover, the magnitude of roughness reduction was comparable between the fluoride and nano-silver toothpaste groups. Evidence regarding the effects of nano-silver-containing materials on enamel surface roughness remains limited [4,17,19]. Nevertheless, available studies suggest that silver nanoparticles may positively influence enamel surface characteristics. Wu *et al.* [17] evaluated the effect of silver nanoparticles on artificial enamel caries developed in a Streptococcus mutans biofilm model and reported a reduction in enamel surface roughness following treatment. Similarly, fluoride-based remineralization therapies have been shown to improve enamel surface characteristics. Dionysopoulos *et al.* [16] reported that topical fluoride application significantly increased enamel microhardness after bleaching, whereas bleaching procedures have been associated with reductions in enamel microhardness and alterations in surface morphology [16, 18]. In addition, China *et al*. [18] demonstrated that fluoride gels improved the surface characteristics of bleached enamel. Although the exact mechanism by which nano-silver contributes to enamel remineralization has not yet been fully elucidated, previous evidence suggests that silver nanoparticles may enhance enamel resistance to demineralization while simultaneously exerting antimicrobial activity against cariogenic microorganisms [6,19-20]. Their small particle size and large surface area may facilitate interactions with demineralized enamel surfaces and contribute to the preservation of enamel structure [19,21,]. However, further mechanistic studies are required to better understand the role of nano-silver in the remineralization process.

From a clinical perspective, the comparable performance of nano-silver and fluoride toothpaste observed in the present study suggests that nano-silver-containing dentifrices may represent a promising adjunctive approach for the management of enamel white spot lesions. In addition to their potential remineralizing effects, nano-silver-containing products may provide additional antimicrobial benefits that could contribute to caries prevention [6,22]. Several limitations of this study should be acknowledged. First, the in vitro design does not fully replicate the complex biological conditions of the oral environment, including salivary flow, acquired pellicle formation, dietary influences, oral microbiota, and fluctuations in pH. Second, only one commercially available nano-silver toothpaste and one fluoride toothpaste were evaluated. Third, the sample size was relatively limited, and the duration of the experimental period was restricted to 15 days. Finally, only microhardness and surface roughness were assessed, while other indicators of remineralization, such as mineral content analysis and microscopic evaluation, were not investigated. Therefore, further *in vivo* studies and randomized clinical trials are warranted to confirm the long-term effectiveness and clinical applicability of nano-silver-containing toothpastes in the prevention and management of enamel white spot lesions.

Conclusion

Within the limitations of this *in vitro* study, nano-silver toothpaste demonstrated a remineralization potential comparable to that of conventional fluoride toothpaste in the management of enamel white spot lesions. Both interventions significantly increased enamel microhardness and reduced surface roughness following demineralization. These findings suggest that nano-silver-containing toothpaste may represent a promising alternative to fluoride toothpaste for promoting enamel remineralization. Further *in vivo* studies and randomized clinical trials are required to confirm its long-term clinical effectiveness.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Maden EA, Altun C, Polat GG, Basak F. The In vitro Evaluation of the effect of xyliwhite, probiotic, and the conventional toothpastes on the enamel roughness and microhardness. Niger J Clin Pract. 2018; 21: 306-311.

[2] Silva AVC, Teixeira JA, Melo PC, Lima MGS, Mota CC, Lins EC, et al. Remineralizing potential of nano-silver-fluoride for tooth enamel: an optical coherence tomography analysis. Pesqui Bras Odontopediatria Clín Integr. 2019; 19: e4002.

[3] Behrouzi P, Heshmat H, Hoorizad Ganjkar M, Tabatabaei SF, Kharazifard MJ. Effect of Two Methods of Remineralization and Resin Infiltration on Surface Hardness of Artificially Induced Enamel Lesions. J Dent (Shiraz). 2020; 21: 12-17.

[4] Akyildiz M, Sönmez IS. Comparison of Remineralising Potential of Nano Silver Fluoride, Silver Diamine Fluoride and sodium fluoride varnish on artificial caries: An in vitro study. Oral Health Preventive Dent. 2019; 17: 469-477.

[5] Teixeira JA, Silva AVC, Elias Dos Santos Júnior V, Correia de Melo Júnior P, Arnaud M, Goretti Lima M, et al. Effects of a new nano-silver fluoride-containing dentifrice on demineralization of enamel and streptococcus mutans adhesion and acidogenicity. J Dent. 2018; 10: 1155.

[6] Ahmed F, Prashanth ST, Sindhu K, Nayak A, Chaturvedi S. Antimicrobial efficacy of nanosilver and chitosan against Streptococcus mutans, as an ingredient of toothpaste formulation: An in vitro study. J Indian. 2019; 37: 46-54.

[7] Amaechi BT, Higham SM, Edgar WM. Factors affecting the development of carious lesions in bovine teeth in vitro. Arch Oral Biol. 1998; 43: 619-628.

[8] Heshmat H, Ganjkar MH, Miri Y, Fard MJ. The effect of two remineralizing agents and natural saliva on bleached enamel hardness. Dent Res J (Isfahan). 2016; 13: 52-57.

[9] Tantbirojn D, Huang A, Ericson M, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent.2008; 36: 7.

[10] Alkattana R, Lipperta F, Tangc Q, George J, Eckertc Andoa M. The influence of hardness and chemical composition on enamel demineralization and subsequent remineralization. J Dent. 2018; 75: 34-40.

[11] Zhi Q H, Lo EC M, Kwok ACY. An in vitro study of silver and fluoride ions on remineralization of demineralized enamel and dentine. Australian Dent J. 2013; 58: 50–56.

[12] El-Desouky D, Hanno A, Dowidar K, Hamza SA, Lubna M, et al. Evaluation of the Anticariogenic Effect of Nano Silver Fluoride on Demineralization of Enamel in Primary Teeth (an in Vitro Study). Alexandria Dent J. 2021; 46: 153-159.

[13] Maia AC, Mangabeira A, Vieira R, de Almeida Neves A, Lopez RT, Pires TM, et al. Experimental composites containing quaternary ammonium methacrylates reduce demineralization at enamel-restoration margines after cariogenic challenge. Dent Mater. 2019, 35: e175-e183.

[14] Vieira Costa e Silva A, Teixeira JA, Mota CCBO, Clayton Cabral Correia Lins E, Correia de Melo Júnior P, Goretti de Souza Lima M, et al. In Vitro morphological, optical and microbiological evaluation of nanosilver fluoride in the remineralization of deciduous teeth enamel. Nanotechnology. 2018; 7: 509-520.

[15] Basir L, Meshki R, Aghababa H, Rakhshan V. Effects of three commercial toothpastes incorporating "chitosan, casein phosphopeptide-amorphous calcium phosphate, sodium monofluorophosphate, and sodium fluoride" on remineralization of incipient enamel caries in the primary dentition: A preliminary in vitro study. Dent Res J (Isfahan). 2020; 17: 433-438.

[16] Dionysopoulos D, Koliniotou-Koumpia E, Tolidis K, Gerasimou P. Effect of Fluoride Treatments on Bleached Enamel Microhardness and Surface Morphology. Oral Health Prev Dent. 2017; 15: 169-175.

[17] Wu R, Zhao Q, Lu S, Fu Y, Yu D, Zhao W. Inhibitory effect of reduced graphene oxide-silver nanocomposite on progression of artificial enamel caries. J Appl Oral Sci. 2018; 27: e20180042.

[18] China AL, Souza NM, Gomes Ydo S, Alexandrino LD, Silva CM. Effect of fluoride gels on microhardness and surface roughness of bleached enamel. Open Dent J. 2014; 8: 188-193.

[19] Pushpalatha C, Bharkhavy KV, Shakir A, Augustine D, Sowmya SV, Bahammam HA, et al. The Anticariogenic Efficacy of Nano Silver Fluoride. Front Bioeng Biotechnol. 2022; 10: 931327

[20] Sabbah A, Helwa I. Efficiency of nano siver fluoride on prevention of dental caries on intact enamel surface assessed by EDX-Analysis (in vitro study). Egyptian J Dent. 2022; 68: 63-69.

[21] Azheen A, Hadi I, Amin K. Effect of nanosilver mouthwash on prevention of white spot lesions in patients undergoing fixed orthodontic treatment-arandomized double-blind clinical trial. J Detal Sci. 2022; 17: 249-255.

[22] Devi C, Chandrasekhar R, Vinay C, Uloopi KS, RojaRamya KS, Ramesh MV. A Randomized Controlled Trial Evaluating the Effectiveness of Nanosilver Fluoride in Arresting Dental Caries in School Children. Int J Clin Pediatr Dent. 2023; 16: 667-670.