EGYPTIAN
DENTAL JOURNAL
www.eda-egypt.org

Vol. 70, 3983:3992, October, 2024

Print ISSN 0070-9484 • Online ISSN 2090-2360

Conservative Dentistry and Endodontics

Submit Date : 05-08-2024 • Accept Date : 14-09-2024 • Available online: 05-10-2024 • DOI : 10.21608/edj.2024.310027.3151

EFFECT OF SILVER DIAMINE FLUORIDE, NANOSILVER FLUORIDE, AND SODIUM FLUORIDE ON DENTIN SURFACE TOPOGRAPHY UNDER EROSIVE CHALLENGE: IN VITRO STUDY

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ABSTRACT

Purpose: This in vitro study used a scanning electron microscope (SEM) to assess and compare the occlusion of dentinal tubules following dentin treatment with silver diamine fluoride (SDF), nanosilver fluoride (NSF), or sodium fluoride (NaF).

Materials and methods: Forty sound human premolar teeth had occlusal enamel wet-ground to yield flat dentin surfaces. A 2 mm thick dentin disc was obtained by cutting a parallel cut above the cementoenamel junction. All dentin discs were submerged in the demineralizing solution to produce artificial erosive lesions. The remineralizing agent was applied for three minutes to each of the three treatment groups (sodium fluoride, nano silver fluoride, and silver diamine fluoride). For a week, pH cycling was carried out on all dentin specimens. The environmental scanning electron microscope was used to analyze the specimens' surfaces following treatment. An occlusion scoring system (ranging from 1 to 5) was used to evaluate the degree of dentinal tubule occlusion.

Results: The demineralized dentin surface topography revealed that the smear layer and plugs had been completely removed from the dentinal tubules, which had opened orifices. No Statistically significant difference in dentinal tubule score was found between NSF (mean 1.2±0.45) and SDF (mean 1±0) groups. The NaF group reported a mean of (3.6±0.55), while the negative control group had the least dentinal tubule occlusion (mean 4.8±0.45).

Conclusions: The dentinal occluding effect of Nanosilver fluoride was comparable to that of Silver Diamine Fluoride. In contrast to NaF, both SDF and NSF were able to resist the acidic challenges.

KEYWORDS: Dentinal tubules occlusion, Dentin Hypersensitivity, Silver Diamine Fluoride, nanosilver particles.

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INTRODUCTION

Several people frequently suffer from dentin hypersensitivity, characterized by sharp, brief pain driven by mechanical, chemical, or thermal stimuli. Dentin hypersensitivity (DH) is caused by dentinal fluid movement in the patent dentinal tubule, which can frequently be triggered by abrasion, attrition, or erosion [1].

One of the primary contributors to the onset of dentin hypersensitivity is dental erosion. Dental erosion has become more prevalent because of the greater consumption of acidic beverages. The current theory behind managing DH is that dentin hypersensitivity might be efficiently reduced by occlusion of the opened dentinal tubules. This would decrease dentin permeability, which would in turn diminish dentinal fluid flow and, ultimately, lessen DH [1].

Nevertheless, most dentin desensitizers do not exhibit prolonged effects due to the re-exposure of the obstructed dentinal tubules on the dentin surface that occur when dietary stressors are encountered [2].

Since 1943, sodium fluoride (NaF), a potent remineralizing agent, has been utilized extensively as a desensitizer. Among the dentin desensitizers introduced to the market, Silver Diamine Fluoride (SDF) has demonstrated promise in preventing dental caries and reducing dentin hypersensitivity [3]. Nevertheless, SDF results in gingival irritation, a bitter metallic taste, and discoloration of the teeth and soft tissues [4]. Nanosilver Fluoride (NSF) was introduced by Targino et al which has combined the antimicrobial properties of nanosilver particles and the remineralizing effect of fluoride [5].

Combining the advantages of dentin remineralization and stability in erosive oral environment challenges would be necessary to regulate dentin hypersensitivity effectively.

This study aims to assess and compare the effect of sodium fluoride, nanosilver fluoride, and silver diamine fluoride in occluding dentinal tubules under erosive challenges. The null hypothesis was that there is no difference between sodium fluoride, nanosilver fluoride, and silver diamine fluoride on the occlusion of dentinal tubules.

MATERIALS AND METHODS

Ethical Approval:

The current study has been approved by The Institutional Review Board Organization IORG0010868; Research Number: *IRB00012891#62*.

Materials:

This in vitro study used Silver Diamine Fluoride (SDF), Nanosilver Fluoride (NSF), and 5% Sodium Fluoride (NaF). (Table 1)

TABLE (1) Descriptive details of the materials used in the study

	Manufacturer	Composition
Silver Diamine Fluoride (SDF)	Advantage Arrest, Elevate Oral Care, West Palm Beach, Fl, USA	The solution ($AgNH_2F$) contains 25% silver 62% water, 5% fluoride, 8% amine, PH =10.
Nanosilver Fluoride (NSF)	Nanosilver fluoride was prepared by Nanotech company, Egypt.	The NSF was manufactured following the procedure outlined by Targino et al [5].
5% Sodium Fluoride (NaF)	Sodium Fluoride powder (NaF) purchased from PIOCHEM, Egypt Cas no.7681-49-4. 5% NaF was prepared in the Pharmacy Faculty at Ahram Canadian University.	0.5 g of NaF was dispersed in 1000 ml of distilled water.

Methods:

To assess the impact of the three remineralizing agents on dentin surface topography, an in vitro experimental investigation was designed.

Study Design:

This in vitro study used 40 sound, non-carious human premolar teeth collected from the outpatient clinic of the surgery department, faculty of Dentistry, Ahram Canadian University, Egypt. Dentin discs (n=40) measuring 2 mm in thickness were obtained, and the specimens were then split into 4 groups (n = 10); Group C represented the negative control group, Group SDF referred for silver diamine fluoride, Group NSF for nanosilver fluoride, and Group NaF for sodium fluoride.

Nanosilver particles Characterization

A transmission Electron Microscope (TEM) was used to observe the particle size, shape, and distribution of nanosilver particles. The Microscopic analysis was performed on JEOL JEM-2100 high-resolution TEM at an accelerating voltage of 200 kV.

Samples Preparation:

Forty sound, non-carious human premolar teeth were kept at room temperature in distilled water until the conduction of the study. The occlusal enamel was wet-ground at a low speed using an isomet (BesQual, NY 11373, USA) to provide a flat dentin surface up to the dentino-enamel junction. A parallel cut was then made above the cementoenamel junction to produce a dentin disc of 2 mm in thickness (Fig. 1). A digital micrometer (Tri circle, Shanghai, China) was utilized to assess the thickness of every dentin disc (Fig. 2 b). To assist in the designation of the coronal surface, the pulpal side of each disc was marked. To achieve a uniform smooth dentin surface, the occlusal surface of each dentin disc was subjected to a 30-second sandblasting process using 600-grit silicon carbide paper. Each dentin disc had been examined for the presence of microcracks using a magnifying lens (NAZANO-USA). Subsequently, the specimens were placed in sealed containers containing distilled water.

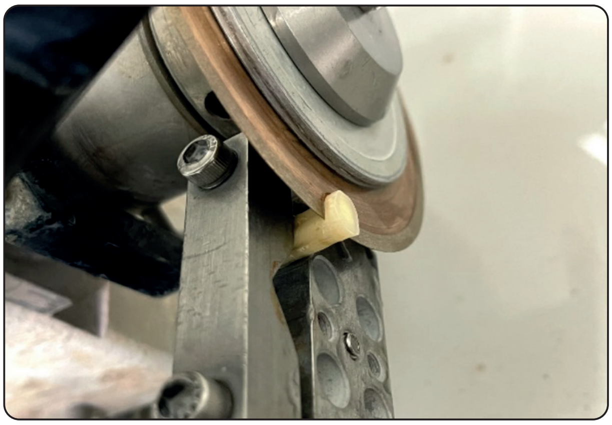

Fig. (1) Water-cooled Isomet low speed was used to wet-grind the occlusal enamel until the dentino-enamel junction to obtain a flat dentin surface. A parallel cut was then made above the cementoenamel junction to produce a dentin disc of 2 mm in thickness.

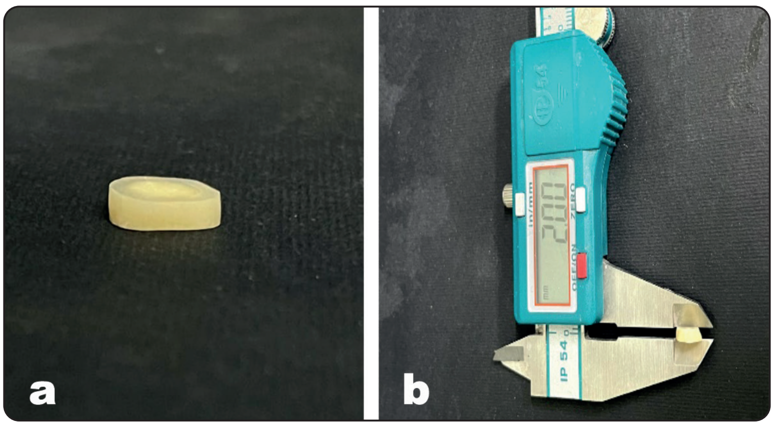

Fig. (2) (a) A dentin specimen of 2 mm thickness. (b)The thickness of each dentin disc was checked using a digital micrometer.

The samples were split into four groups of ten disks each: Group C: Negative control, Group SDF: Silver Diamine Fluoride, Group NSF: Nanosilver Fluoride, and Group NaF: Sodium Fluoride.

Artificial Erosive Lesions Preparation:

For three days at 37^0C, each dentin specimen was submerged in the demineralizing solution. During the demineralization period, the demineralizing solution (2.2 mM $CaCl_2$, 2.2 mM KH_2 PO_4, 0.05M acetic acid, pH 5) was changed daily.

A pH electrode (GE 100 BNC, GHM Greisinger, Germany) linked to a pH meter (GMH 3531, Greisinger, Germany) was used daily to test the pH values of the demineralizing fluids during the demineralization period. A steady pH value of 4.99 and 5.01 for dentin was maintained by adjusting slight elevations with low doses of hydrochloric acid (HCl). To get rid of any extra acids, deionized water was used to wash the dentin specimens [4].

A scanning electron microscope (FEI Company, Netherlands) was used to analyze representative specimens to ensure the opening of the dentinal tubules.

Surface Treatment:

Following a 3-day immersion in the demineralizing solutions, each of the three treatment groups [(NaF), (NSF), and (SDF)] received treatment with the appropriate remineralizing agent. The remineralizing agent was applied using a microbrush onto the specimens' occlusal dentinal surfaces for 3 minutes, after which they underwent pH cycling and were submerged in artificial saliva (0.4 g NaCl, 0.4 g KCl, 0.6 g $CaCl_2$, 0.6 g NaH_2 PO_4, 4 g Urea, 4 g Mucin, 0.0016 g Na_2S, 0.0016 g Mg_2 P_2 O_7 + 1L distilled water at pH 7). Without any surface treatment, the negative control group underwent pH cycling.

pH cycling:

For one week, each dentin specimen was exposed to a pH cycling. The daily cycle consisted of two hours of remineralization sandwiched by three hours of demineralization.[4] The specimens were immersed in artificial saliva for an entire night following the daily cycle. The three treatment groups (SDF, NSF, and SF) received treatment thrice daily at a temperature of 37°C. The treatments were administered before the first demineralization and before and after the second demineralization. Before inserting the specimens into each phase, the demineralizing and remineralizing solutions' pH values were evaluated. All specimens were immersed in the solutions by placing them in a glass jar during pH cycling to replicate the oral environment [6].

Assessment of Dentin Surface Topography:

Specimens were subjected to surface analysis to assess the degree of dentinal tubule occlusion after treatment using the Environmental Scanning Electron Microscope Model Quanta 250 FEG (Field Emission Gun) with accelerating voltage 30 K.V.

To assess dentinal tubule occlusion, the dentin discs were first dried in a desiccator for twenty-four hours and, after that, placed on aluminum stubs, sputter coated with gold, and examined under an ESEM at magnifications of ×1000 and ×2000. The assessment of the impact of the remineralizing agent on the degree of dentinal tubule occlusion in the SEM images was conducted by two blinded evaluators utilizing an occlusion scoring system (ranging from 1 to 5) [7].

1 = 100% of dentinal tubules are completely occluded.

2 = 75% of dentinal tubules are completely occluded.

3 = 50% of dentinal tubules are completely occluded.

4 = 25% of dentinal tubules are completely occluded.

5 = no dentinal tubule occlusion.

The following formula was used to calculate the percentage of fully occluded tubules: Number of dentinal tubules that are fully occluded following treatment / Number of opened dentinal tubules before any kind of therapy X100 [5]. The computer software used for the image analysis, the Image J-Fiji program, binarizes the image such that the black and white surface elements (black: opened dentinal tubules, white: occluded dentinal tubules) may be differentially numbered [8].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 20 was utilized for data administration and statistical analysis. To consolidate numerical data, the mean and standard deviation were calculated. Using the Kolmogorov-Smirnov and Shapiro-Wilk tests, the data were examined for normality in addition to examining the data distribution. Using ANOVA, comparisons between groups were made for normally distributed numeric variables. Using the chi-square test, categorical data written as numbers and percentages were compared. P-values have been double-sided. P-values less than or equal to 0.05 were deemed to be statistically significant.

RESULTS

TEM Results:

The crystal structure of the manufactured nano silver particles was confirmed using TEM investigation, which revealed particles with sizes of 23 to 40.4 nm, as illustrated in Fig. 3.

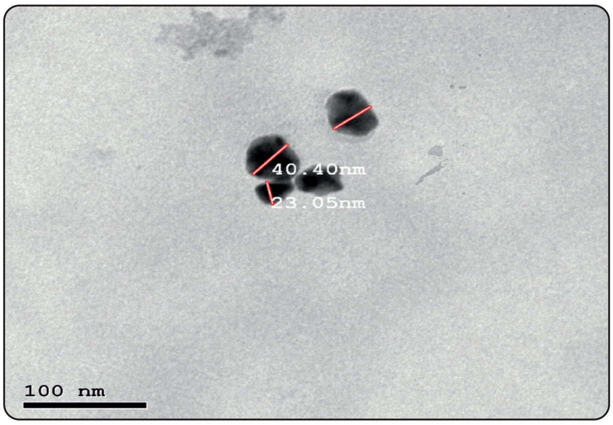

Fig. (3) TEM nanograph for the prepared nanosilver particles.

SEM Results:

Dentin Surface Topography:

Demineralized dentin surface topography demonstrated complete elimination of the smear layer and smear plugs from dentinal tubules that appeared wide with opened orifices (Fig. 4). Dentin treated with SDF, NSF, and NaF showed dentinal tubules occlusion of varying degrees. The least dentinal tubule occlusion was recorded in the negative control group (untreated group) (Fig. 5). The NaF group showed a decrease in the diameter of the dentinal tubules along with partial obliteration (Fig. 6). Complete obliteration of dentinal tubules was obvious in SDF and NSF groups (Figs. 7 and 8). The SDF group showed a thick elevated membrane-like coating on the dentin surface, with occluded all dentinal tubules (Fig. 7). The NSF group showed occluded most of the dentinal tubules with crystal-like deposits in addition to multiple elevated deposits on the dentin surface (Fig. 8).

Frequency of dentinal tubules score

All samples of the demineralized dentin group recorded a score of 5, while all samples of SDF recorded a score of 1. In the NaF group, 60% of the samples scored 4, and 40% scored 3. In the NSF group, 80% of the samples scored 1, and 20% scored 2. In the negative control group, 80% of the samples scored 5, and 20% scored 4. A statistically significant difference (p=0.000) was observed between the groups.

Mean dentinal tubules score

The greatest value was recorded in the Demineralized dentin (Dem D) group (mean 5±0) and negative control group (mean 4.8±0.45). The difference between Dem D and the negative control groups was not statistically significant.

The NaF group reported a mean of (3.6±0.55). The lowest values were recorded in the NSF group (mean 1.2±0.45) and SDF group (mean 1±0). No significant difference was found between NSF and SDF groups. Statistically, the difference between groups was significant (p=0.000), (Table 1, Fig. 9).

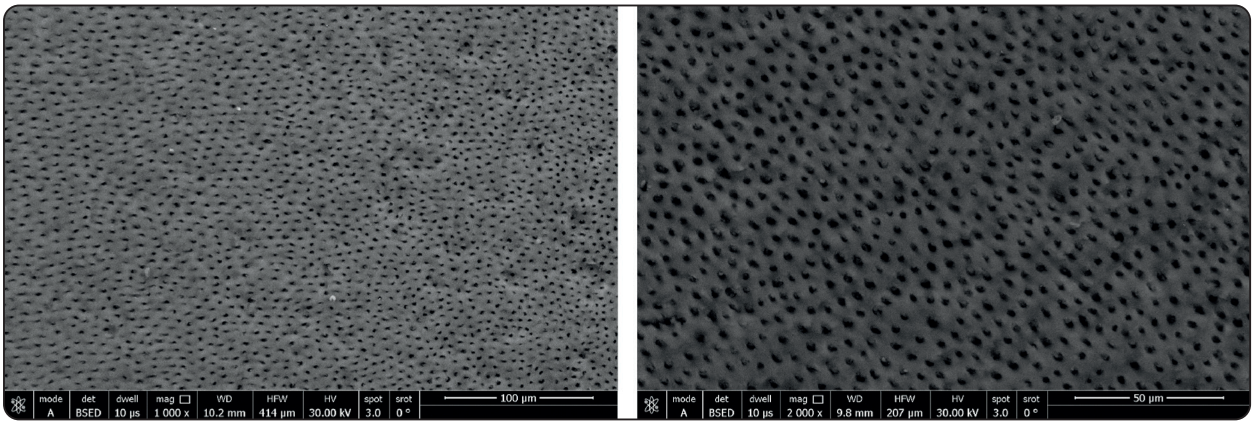

Fig. (4) ESEM showing surface topography of demineralized dentin (X1000 and X2000 Magnifications)

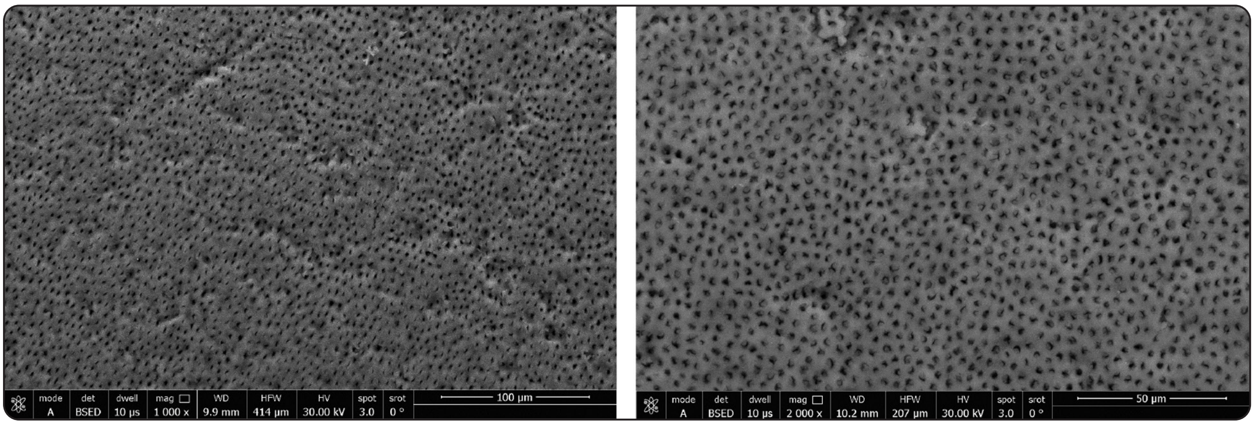

Fig. (5) ESEM showing dentin surface topography of negative control (untreated) group (X1000 and X2000 Magnifications)

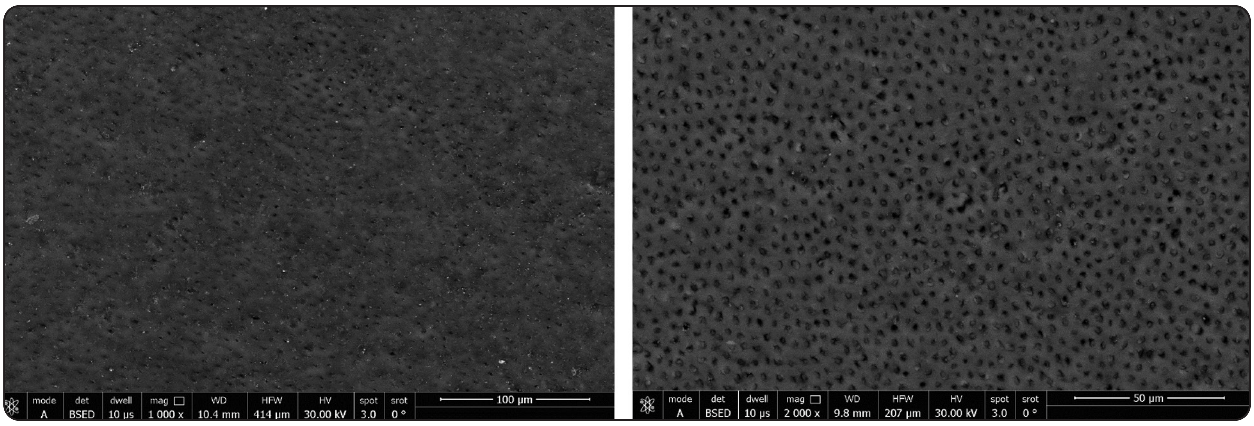

Fig. (6) ESEM showing dentin surface topography of NaF group (Sodium fluoride group) (X1000 and X2000 Magnifications

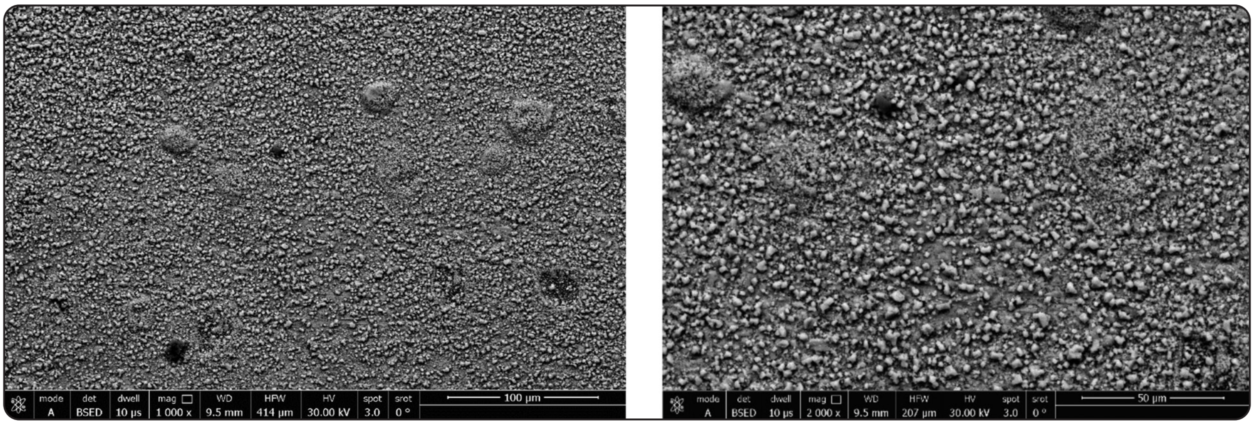

Fig. (7) ESEM showing dentin surface topography of SDF group (Silver Diamine Fluoride group) (X1000 and X2000 Magnifications)

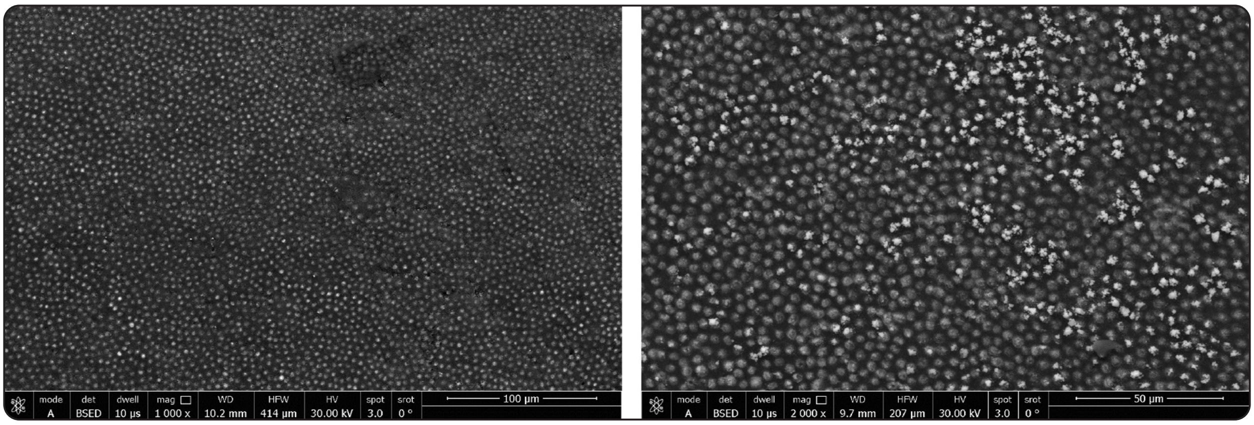

Fig. (8) ESEM showing dentin surface topography of NSF group (Nano silver Fluoride group) (X1000 and X2000 Magnifications)

TABLE (2) Descriptive statistics of mean dentinal tubules occlusion scores and comparison between groups (ANOVA test)

	Mean	Std. Dev	Minimum	Maximum	F value	P value
DemD	5[a]	.00	5.00	5.00	131.68	.000*
NaF	3.6[b]	.55	3.00	4.00		
NSF	1.2[c]	.45	1.00	2.00		
SDF	1[c]	.00	1.00	1.00		
Negative control	4.8[a]	.45	4.00	5.00		

Significance level p≤0.05, *significant

Post hoc test: means sharing the same superscript letters are not significantly different.

Dem D: demineralized dentin; NaF: sodium fluoride; NSF: Nanosilver fluoride; SDF: Silver diamine fluoride.

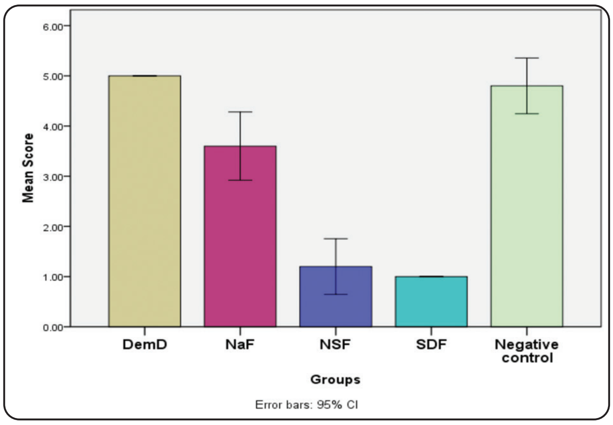

Fig. (9) Bar chart illustrating mean dentinal tubules occlusion scores in different groups

DISCUSSION

Many individuals suffer dentin hypersensitivity (DH), a prevalent condition accompanied by intermittent severe pain. Dentinal fluid movement in the dentinal tubules has been speculated to be induced by external stimuli, which trigger sensory nerve endings and generate dentin hypersensitivity [1,9].

Many approaches have been used to decrease and treat dentin hypersensitivity. At present, the approach to managing dentinal hypersensitivity (DH) relies on the notion that constriction of the opened dentinal tubules can efficiently alleviate dentin hypersensitivity by reducing dentin permeability and dentinal fluid flow, thereby causing a decrease in DH [10].

Fluoride remains the gold standard for both enamel and dentin remineralization, thus NaF was used in the current study as the positive control group [11]. The prepared NaF (5%) was preferred to be used in this study as most of the commercially available varnishes contain tricalcium phosphate or ingredients such as sodium saccharin or xylitol used as a sweetener [11]. Silver diamine fluoride (SDF) is a clear, odorless liquid that causes dentinal tubule occlusion but causes staining of teeth and gingiva[3]. Therefore, the current study aims to assess and compare the impact of SDF and nanosilver fluoride (NSF) on dentinal tubule occlusion. The antibacterial properties of silver nanoparticles (AgNPs) and the remineralizing capabilities of fluoride can be combined in a prospective formulation called nanosilver fluoride (NSF), which doesn't change the color of tissues [12,13].

Ionic silver, fluoride, and ammonia are the ingredients of the silver diamine fluoride (SDF) solution [14]. SDF's fluoride content aids in the production of fluorapatite and remineralization, resulting in strong, acid-resistant dentin. The silver offers the substance antibacterial action and prevents the growth of biofilms [15]. The Food and Drug Administration recently approved the substance to desensitize sensitive teeth [15].

The pH cycling model used was a chemical model to mimic the erosive challenges present in the oral cavity that could cause the erosion of teeth [16]. Nevertheless, this scenario diverges significantly from the actual circumstances because of the lack of microorganisms involved in the demineralization and remineralization mechanisms [16]. Artificial saliva was used as a storage medium in the current study to simulate the human oral environment [17].

The successful creation of artificial erosive lesions was confirmed by ESEM images of demineralized dentin (Fig. 4) that revealed patent dentinal tubules and recorded a score of 5. Untreated dentin (Fig. 5) showed 25% occluded dentinal tubules which could be due to the effect of artificial saliva used as a storage medium, in addition to the dynamic Ph cycling consisting of alternating demineralization and remineralization processes.

The SDF group recorded a score of 1. In other words, 100% of the dentinal tubules of the SDF group were completely occluded in addition to the formation of a thick elevated layer on the dentin surface (Fig. 7). This layer could potentially be attributed to the deposition of insoluble complexes produced by SDF upon blending with artificial saliva [8].

These findings confirm that SDF can resist acidic erosive challenges. However, the deposits that could be formed in the dentinal tubule's lumen of the NaF group failed to resist the acidic erosive challenges. For this reason, 50% of the dentinal tubules appeared unoccluded in the NaF group (Fig.6).

The ESEM results of the NSF group revealed crystal-like deposits within the tubule lumen (Fig. 8). The deep infiltration of the nano-sized silver particles (of maximum size 40 nm) into dentinal tubules may be of great significance since dentinal tubules exhibit diameters of about 2.5 μm.

As there is no statistically significant difference in dentinal tubule score between SDF and NSF groups, the proposed null hypothesis was not rejected. The authors suggest using NSF as an alternative to SDF if esthetics is an important concern.

The findings of this in vitro study have to be seen in the light of some limitations such as short pH cycling, and the absence of bacteria. Further studies are recommended to evaluate the dentinal occluding effects of NSF and SDF in the presence of bacterial challenges for longer durations.

CONCLUSIONS

Under the limitations of the current in vitro study, the findings suggest that the occlusion impact of Nanosilver fluoride on the dentinal tubule is comparable to that of Silver Diamine Fluoride. In contrast to NaF, both SDF and NSF were able to withstand the acidic challenges for one week.

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