EVALUATION OF REMINERALIZING POTENTIAL OF NANO SILVER FLUORIDE VARNISH ON ENAMEL CARIES LIKE LESIONS IN PRIMARY TEETH

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ABSTRACT

INTRODUCTION: Dental caries is a preventable chronic disease. Topical fluorides have been commonly used to manage caries. Nano silver fluoride is promising material in prevention and remineralization of early enamel caries in primary teeth.
OBJECTIVES: To evaluate, in-vitro, the remineralizing potential of nano silver fluoride on enamel caries-like lesions in primary teeth in comparison to silver diamine fluoride and sodium fluoride varnish using energy dispersive x-ray spectroscopy (EDX).
MATERIAL AND METHODS: Thirty-Two extracted or exfoliated caries free primary teeth were collected and coated with nail varnish leaving squares of 4x4 mm exposed enamel on labial surfaces. Caries like lesions were created by immersion in demineralizing solution for 4 days. Teeth were divided into four groups, group I (n=8) treated with nano silver sodium fluoride (NSSF), group II (n=8) treated with 38% silver diamine fluoride (SDF), group III (n=8) treated with 5% sodium fluoride varnish (NaF) and group IV (n=8) left untreated (negative control). All groups were subjected to pH cycling for 10 days. Specimens were evaluated quantitatively using EDX through elemental analysis of Calcium (Ca), Phosphorus(P), Fluoride(F) and Calcium Phosphate ratio(Ca/P) at baseline, after demineralization and after pH cycling. Data were recorded and statistically evaluated. Significance was inferred at P-value <0.05
RESULTS: Using Paired t-test, mean Ca and P increased significantly in the three groups (P=0.01) with no statistical significant difference between them. Using Wilcoxon signed rank test, mean F increased significantly in treated groups of NSSF, SDF and NaF (P= (0.002), (0.003) (0.01)) respectively. Using Kruskal Wallis test, NSSF group showed significantly the highest mean F ion content after remineralization (P=0.02).
CONCLUSION: Nano silver fluoride is as effective as SDF and NaF varnish in remineralization of enamel caries like lesions in primary teeth. Moreover, nano silver fluoride showed a notable increase in F compared to SDF and NaF varnish.

KEYWORDS: Nano silver fluoride, silver diamine fluoride, fluoride varnish, artificial caries, primary teeth.
RUNNING TITLE: Remineralizing potential of nano silver fluoride

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INTRODUCTION

Dental caries is considered the most prevailing oral cavity disease. It occurs as a result of tooth surface demineralization caused by organic acids' action produced by bacterial fermentation of carbohydrates and degradation of organic matrix (1). Demineralization is a dynamic process that can be reversed if detected early. Dental caries progression takes place, when demineralization periods occur more frequently than remineralization periods (2). Mineral loss caused by progression of the disease produces visual alterations in teeth surface, beginning with the subclinical stage (white spots) and progressing to cavitations (3). The first stage of enamel carious lesions with sound enamel surface as well as total mineral loss from the subsurface, is known as an incipient carious lesion (ICL).

Management of ICL necessitates non-invasive methods like remineralization with proper materials or using resin-based agents (4).

In recent years, restorative dentistry has directed its attention to a more conservative approach, with remineralization techniques emerging as the most popular and effective way to restore missing tooth structure. Early detection, conservation, and non-restorative treatment of early caries saves time and money in the dental office, as well as patient's pain (5). Therefore, fluoride application is now used to prevent as well as arrest caries (6). Fluoride varnishes provide several advantages over other modalities of topical fluoride treatments because varnishes are effective in caries prevention, relatively safe, acceptable and easily applied to tooth surfaces (7).

To improve the caries-arresting effects, products with increased fluoride concentrations have been developed. Due to its effectiveness in prevention of dental caries progression, a metal ion based topical fluoride preparation known as silver diamine fluoride (SDF) has gotten a lot of attention. In 2014 the US food and drug administration (FDA) approved the SDF product. The most widely used concentration of SDF is 38% that incorporates 44,800 ppm F. It has been reported that 38% SDF is effective to prevent the progression of dental caries by 65.9% (8).

Silver diamine fluoride is the first material to combine the remineralizing capacity of sodium fluoride with the antibacterial effect of silver nitrate. It acts through the formation of silver phosphate on outer tooth structure, uptake of fluoride in dentin and dentinal tubules blockage by silver precipitates. The mineral content of the tooth improves the resistance of the peri- and intertubular dentin to acid demineralization (9). Nevertheless, the main disadvantage of SDF is the dark black staining of carious tissues which caused by the oxidation of silver ions present in its formulation, as well as ulceration and painful staining of gingival tissues. Conversely, stains of soft tissues caused by SDF are commonly reversible (10).

With the advent of nanotechnology, alternative nano silver based preparation, has been introduced to solve black staining problem. According to recent studies, nanotechnology may provide innovative preventive dentistry approaches, especially in remineralization of submicrometre-sized tooth decay (11-13). These preparations are becoming increasingly popular as excellent biocidal materials. The action of nano-silver is through the contact with microorganisms. Silver nanometre scale enables a broad contact area to the bacteria (14). Also, the nanoparticles have the ability for penetration to the bacterial cell wall, interference with the replication of DNA and inhibition of cellular respiration by impairing direct and indirect lipid peroxidation (15). Moreover, when they get in the bacterial cell, they continuously release silver ions, produce free radicals, cause oxidative stress and further enhance bactericidal activity (14). Therefore, there is a sound rationale for the addition of silver nanoparticles to fluoride varnishes in an attempt to acquire preventive and antimicrobial characteristics without the black staining of tooth, as silver diamine fluoride does. Nano silver fluoride (NSF) a new formula was introduced to be an effective anti-caries material (16).

Dos Santos et al (9), have reported in their trial the efficacy of Nano-silver containing fluoride varnishes in arresting caries in children. Also, Nozari et al (17) compared the remineralization ability of NSF, Nano-hydroxyapatite serum and NaF varnish on enamel of primary anterior teeth using Surface Microhardness (SMH) and concluded that NSF had the greatest remineralization efficacy amongst the three materials.

Different studies have compared the effects of NSF with SDF on permanent enamel lesions (18, 19). While, there has not been a lot of research comparing the remineralizing effect between them on enamel caries in primary teeth. Thus, the present study aimed at evaluating the effect of nano silver fluoride on remineralization potential of enamel caries like lesions in comparison to silver diamine fluoride and 5% sodium fluoride varnish in primary teeth in terms of mineral deposition. The null hypothesis is that there will be no difference in remineralizing effect between the three remineralizing agents used in the present study.

MATERIALS AND METHODS

The comparative experimental in vitro study was approved by the ethics committee in the Faculty of Dentistry Alexandria University (IORG0008839). The minimal sample size was estimated based on earlier studies (20, 21). According to the following assumptions: alpha error= 5% and study power= 80%, sample size was estimated to be 6 per group and this was increased to 8 to make up for laboratory processing errors. The total sample size required to detect the effect of various fluoride varnishes on remineralization of caries-like lesions was 32. The sample size was calculated using IBM SPSS version 17.0 software (22).

Thirty-two primary teeth (incisors and molars) exfoliated or extracted for orthodontic reasons were collected from the out-patient clinics of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University and from the Ministry of Health Hospitals in Alexandria. A magnifying lens was used to examine the selected teeth ensuring that they fulfilled the inclusion criteria of having no caries, cracks, restorations or any developmental defects. Teeth were kept in deionized water till required for use.

Sample preparation

Teeth were cleansed using fluoride free pumice then washed with distilled water and air-dried. A 4×4 mm square of self-adhesive tape was stuck at the center of the labial surface of each tooth. All surfaces of the teeth were coated with a layer of acid proof nail varnish. Then, the adhesive tapes were removed exposing only a small window of standardized enamel in all the specimens.

Caries like lesions formation

All teeth were incubated at 37°C in demineralizing solution (2.2 mM Calcium chloride ($CaCl_2$), 2.2 mM Potassium dihydrogen phosphate (KH_2PO_4), 0.05 M Acetic acid (CH_3COOH) and 1 M Potassium hydroxide (KOH) was used to optimize pH to 4.4), 10ml for each specimen, for 96 hours to induce early enamel caries lesion without cavitation (23). Instead of using carious teeth with varied amounts of demineralization, an artificial caries model was used to simulate the demineralization process that could occur in the tooth structure and establish a generalized baseline for the different specimens. The demineralizing solution was changed every 48 hours to avoid its depletion, saturation or the accumulation of enamel dissolute products (24). Then, the specimens were removed from the artificial caries system and washed with deionized water.

Sample grouping and randomization

Teeth were divided randomly using permuted block technique into 4 groups; each group consists of 8 specimens. Group I: was treated by nano silver sodium fluoride varnish. Group II: was treated by 38% silver diamine fluoride (Advantage Arrest™, Elevate Oral Care, LLC, West Palm Beach, FL. USA). Group III: was treated by 5% sodium fluoride varnish (Enamel Pro® varnish™, Premier® Dental Products Company). Group IV: no treatment (negative control group).

Nano silver fluoride preparation

The preparation of 5% nano silver sodium fluoride (NSSF) was clarified by Haghgoo et al. (26) using weight dilution method. 10 ml of 22,600 ppm of 5% Sodium fluoride varnish (Enamel Pro® varnish™) has been added to 0.5 grams of Silver nano-particle powder (US- Research-Nanomaterials, Ag 99.9%, 80-100nm, metal basis) in a brown bottle that is light proof. Vigorous stirring was done using speed mixer machine (in Dental Biomaterials Department, Faculty of Dentistry, Alexandria University) to get even distribution of Nano-silver particles (14).

Treatment Procedure

For group I: Nano silver sodium fluoride varnish was applied with microbrush to enamel specimens for 2 minutes then washed with deionized water for approximately 1 minute according to Akyildiz et al (19).

For group II: Silver diamine fluoride was applied with microbrush to enamel specimens, then allowed to soak in for 1 minute. Excess was removed with a cotton pellet. Then it let to become dry completely for 1 minute according to Yu et al (20).

For group III: Enamel Pro® vanish (5% sodium fluoride varnish) was applied with microbrush to enamel specimens consistent with the instructions of manufacturers.

For group IV: Negative control group, No treatment.

pH cycling model

Continuous cycles were run daily for 10 days, each cycle involved three hours of demineralization twice a day with two hours of remineralization in between. This model was designed according to Malekafzali et al (27) in order to mimic as closely as possible the dynamic variations in mineral loss and gain of the acid cariogenic challenge during the natural caries process in the oral cavity. Moreover, this model had the purpose of preserving the enamel surface layer and create a sub-surface lesion that closely imitate the natural incipient carious lesion which occurs in vivo. Specimens were placed overnight in remineralizing solution (1.5 mM calcium chloride($CaCl_2$), 0.9 mM sodium dihydrogen phosphate (NaH_2PO_4) and 0.15 M of potassium chloride (KCL)was used to optimize pH to 7.0 (25). Both solutions (demineralizing, remineralizing) were prepared at Faculty of Pharmacy, Alexandria University and fresh solutions were used for each cycle in separated containers for each group during the experimental periods to prevent cross reaction of solutions (27). All specimens have been rinsed with distilled water and prepared for evaluation.

Energy dispersive x-ray spectroscopy (EDX) evaluation

Each specimen was mounted on copper stub and analyzed using EDX (Jeol JSM-IT200 InTouchScope™ Scanning Electron Microscope. Faculty of Science, Alexandria University). Elemental distribution of calcium (Ca), phosphorus (P) and fluoride (F) ions in mass% of enamel were determined in the form of peaks on a graph with their corresponding readings. The Ca and P content were converted into Ca/P ratio for each group. **(Figure 1)**

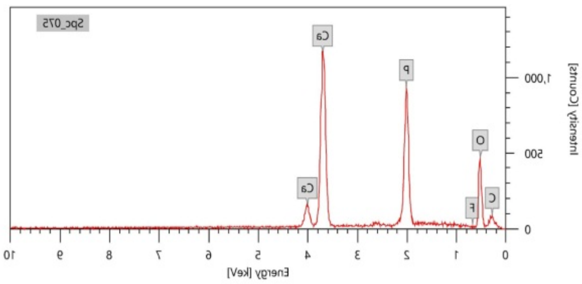

Figure (1): Analysis of Ca, P and F using EDX

Statistical analysis

Data were examined using IBM SPSS software for Windows (Version 23.0). Data were reviewed to check for any errors during data entry. Normal distribution of data was analyzed using descriptive statistics, plots (histogram and box plots), and normality tests. Means and standard deviation (SD) were calculated for all variables, and percent change was calculated using the following equation:

$$\frac{\text{value after remineralized enamel} - \text{value of demineralized enamel}}{\text{value of demineralized enamel}} \times 100.$$

Comparisons of ion concentrations between the four study groups were done using one-way ANOVA for normally distributed variables (Ca, P, and Ca/P), and Kruskal Wallis test for non-normally distributed variables (percent change in Ca, P, Ca/P, F and F ion concentration) at each measurement point. Comparisons of ion concentrations before and after intervention within each group were done using Paired t-test or Wilcoxon signed rank test according to the variable normality. Significance was inferred at P-value <0.05.

RESULTS

At baseline and after demineralization, there was no statistically significant difference between all groups in mean calcium ion content (P =0.76). By comparing the three study groups after remineralization, there was no statistically significant difference between them in mean Ca ion content. However, they were significantly different in comparison to group IV (negative control) (P<0.001). Within groups comparison, the mean Ca ion content showed statistically significant difference before and after intervention in the three study groups (P =0.001). The highest mean percent change was in Group I nano silver sodium fluoride (NSSF) (30.83 ± 8.79), followed by Group II Silver Diamine Fluoride (SDF) (mean ± SD= 23.46 ± 5.55) and Group III Sodium Fluoride varnish (NaF) (mean ± SD= 21.05 ± 6.67) with no statistically significant difference between them. **(Table 1)**

Table (1): Mean Calcium ion content before and after intervention and percentage change among the study groups

Ca	Group I (NSSF)	Group II (SDF)	Group III (NaF)	Group IV (Control)	Test Value (P value)
	Mean ± SD				
Sound enamel [1]	35.45 ± 2.96	36.13 ± 2.44	35.16 ± 3.14	4.50 ± 3.52	0.39 (0.76)
Demineralized enamel [1]	27.93 ± 2.83	27.51 ± 2.30	28.25 ± 3.12	6.93 ± 2.53	0.35 (0.79)
Remineralized enamel 1	36.35 ± 1.96 a	33.93 ± 2.87 a	34.03 ± 2.37 a	6.57 ± 3.72 b	18.30 (<0.001*)
Paired t-test between demineralized and remineralized enamel (P value)	13.29 (<0.001*)	12.33 (<0.001*)	12.93 (<0.001*)	0.63 (0.55)	
Percent change [2]	0.83 ± 8.79 a	23.46 ± 5.55 a	21.05 ± 6.67 a	1.64 ± 6.11 b	20.53 (<0.001*)

1: One-way ANOVA test was used
2: Kruskal Wallis test was used
*: statistically significant at P- value <0.05
a, b: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level

NSSF: Nano silver sodium fluoride
SDF: Silver diamine fluoride
NaF: Sodium fluoride varnish

At baseline and after demineralization, there was no statistically significant difference between all groups in mean Phosphorus ion content. By comparing the three study groups after remineralization, there was no statistically significant difference between them in mean P ion content. However, they were significantly different in comparison to group IV (negative control) (P<0.001). Within groups comparison, the mean P ion content showed statistically significant difference before and after intervention in the three study groups (P =0.001). The highest mean percent change was in NSSF group (mean ± SD = 20.50 ± 6.37), followed by SDF group (mean ± SD= 17.12 ± 5.02) and NaF group (mean ± SD= 15.53 ± 4.81) with no statistically significant difference between them. **(Table 2)**

Table (2): Mean Phosphorus ion content before and after intervention and percentage change among the study groups

P	**Group (NSSF**	**Group I (SDF)**	**Group II (NaF)**	**Group IV (Contro**	**Test value (P value)**
	Mean ± SD				
Sound enamel [1]	16.51 ± 1.12	16.60 ± 1.17	16.49 ± 1.12	16.89 ± 1.25	0.20 (0.8
Demineralized enamel [1]	14.10 ± 0.51	13.81 ± 1.19	14.24 ± 1.76	14.24 ± 1.76	0.2 (0.8
Re-mineralized enamel [1]	16.97 ± 0.67 **a**	16.17 ± 1.39 **a**	16.42 ± 0.81 **a**	14.16 ± 1.88 **b**	7.29 **(0.001***
Paired t-test between demineralized and remineralized enamel (P value)	9.69 **(<0.001**	9.49 **(<0.001***	11.38 **(<0.001*)**	0.47 (0.66)	
Percent change [2]	20.50 ± 6.37 **a**	17.12 ± 5.02 **a**	15.53 ± 4.81 **a**	-0.60 3.32	18.81 **(<0.001**

1: One-way ANOVA test was used
2: Kruskal Wallis test was used
*****: statistically significant at P- value <0.05
a, b: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level
NSSF: Nano silver sodium fluoride
SDF: Silver diamine fluoride
NaF: Sodium fluoride varnish

Regarding Calcium: phosphate ratio "Ca/P", within groups comparison revealed a statistically significant difference before and after intervention in the three study groups (P =0.001). By comparing the three study groups, NSSF group showed the highest mean of Ca/P after remineralization (mean ± SD= 2.15 ± 0.15) followed by SDF group (mean ± SD= 2.12 ± 0.31) and NaF group (mean ± SD= 2.08 ± 0.19) with no statistical significance between them. **(Figure 2)**

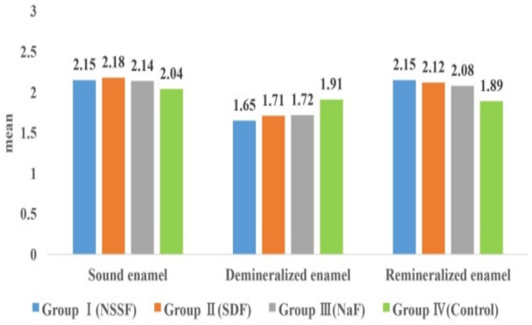

Figure (2): Calcium: Phosphate ratio "Ca/P" in the four study groups before and after intervention

At baseline and after demineralization, there was no statistically significant difference between all groups in mean Fluoride ion content. Within groups comparison showed that the mean Fluoride ion content was statistically significant different before and after intervention in the NSSF, SDF and NaF groups (P = 0.002, 0.003, 0.01) respectively. while, by comparing between groups after remineralization, there was statistically significant difference in mean Fluoride ion content in NSSF group in comparison to the other three groups (P=0.02). Additionally, the percent change was statistically significant different in NSSF group in comparison to the other three groups (P =0.001). However, there was no significant difference in percent change between SDF and NaF groups. **(Table 3)**

Table (3): Mean Fluoride ion content before and after intervention and percentage change among the study groups

F	Group I(NSSF)	Group II(SDF)	Group III(NaF)	Group IV (Control)	Test value (P
	Mean ± SD				
Sound enamel [1]	0.91 ± 0.31	1.02 ± 0.40	1.40 ± 0.64	1.05 ± 0.57	2.51 (0.47)
Demineralized enamel [1]	0.51 ± 0.28	0.38 ± 0.19	0.44 ± 0.17	0.49 ± 0.43	1.20 (0.75)
Re-mineralized enamel [1]	1.01 ± 0.24 **a**	0.72 ± 0.31 **b**	0.98 ± 0.47 **b**	0.45 ± 0.40 **b**	10.06 **(0.02*)**
Wilcoxon signed rank test between de- and re-mineralized enamel (P value)	2.52 **(0.002*)**	2.51 **(0.003*)**	2.51 **(0.01*)**	1.76 (0.08)	
Percent change [1]	41.61 ± 140.33 **a**	118.50 ± 113.50 **b**	141.15 ± 105.14 **b**	-12.98 ± 16.12 **c**	17.41 **.001*)**

[1]: Kruskal Wallis test was used
*****: statistically significant at P- value <0.05

a,b,c: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level
NSSF: Nano silver sodium fluoride
SDF: Silver diamine fluoride
NaF: Sodium fluoride varnish

DISCUSSION

The obtained results of the present study indicated that NSF is as effective as SDF and NaF varnish in increasing Ca, P and Ca/P. While, NSF showed significantly increase in F compared to SDF and NaF varnish. Thus, the null hypothesis was partially rejected.

Remineralization evaluation was done quantitatively by means of elemental analysis for Calcium (Ca), Phosphorus (P), Calcium: Phosphate ratio (Ca/P) and Fluoride (F) using Energy Dispersive X-ray Spectroscopy (EDX), as this device is considered the gold standard to determine the structural analysis of materials (28).

The results of the present study showed that within groups, there was statistically significant increase in mean (Ca, P, Ca/P ratio and F) after intervention in nanosilver, SDF and NaF groups. This attributed to high fluoride content in different treatments modalities groups that had remineralizing effects on enamel surfaces. Thus, indicating their remineralization potential and therapeutic effect on early carious lesions.

By comparing between the study groups, the highest percent increase of Ca and P ions after remineralization were found in nanosilver group. These might be due to the very small particle size of silver nanoparticles which facilitated the penetration of the material into the enamel structure leading to maximizing its effect and increasing deposition of Ca and P ions on demineralized enamel surfaces (29). However, no statistical significant difference was found between nanosilver and the other remineralizing agents.

Meanwhile, the obtained results revealed that the percent increase in F ion content had significantly occured in NSSF group after intervention in comparison to other remineralizing groups. This may be because of the synergistic effect of nanosilver particles to fluoride, thus enhancing remineralization of early carious lesions (30).

The results of the present study are in accordance with Silva et al (31) who concluded that both NSF fluoride and NaF varnish were effective in remineralizing primary teeth enamel through Optical Coherence Tomography (OCT). According to A-scan analysis of images obtained, it was indicated that silver nanoparticles do not impair fluoride action, which could be justified by silver's inherent ionic stability. This outcome is especially critical in primary teeth because of a distinctive property of their enamel; these teeth are more permeable, less thick and less hard than that of permanent enamel (32).

On the other hand, Abo El Soud et al (18) compared in vitro the effects of NSF versus SDF on demineralized enamel surfaces in permanent teeth using EDX and showed that NSF was more efficient than SDF in enamel remineralization. They stated that the possible mechanism of action for arresting enamel caries by either SDF or NSF is inhibition of mineral loss.

In contrast to our results, Akyildiz and Sonmez concluded in their study that NSF had lower remineralization effect in vitro in comparison to SDF and NaF (19). This contradiction might have happened because their study was conducted on permanent teeth, with different formulation of nanosilver (experimental preparation) and another pH cycling model. It is important to emphasize that the sensitivity of the primary teeth has increased to fluoride treatment because their enamel is about 150 times more permeable than permanent teeth allowing the diffusion of fluoride (31).

The overall results of the current study support the remineralizing capacity of nanosilver fluoride on enamel caries of primary teeth. According to Dos Santos study, NSF is simple to use as the treatment procedure does not necessitate the presence of full dental equipment or a clinical setting. It could be applied once a year with the advantage of not staining teeth (9). Also, unlike SDF, NSF has no metallic taste, inexpensive and 5% NSF is eight times economical compared to 38% SDF (14, 33). Therefore, NSF has the potential to be an alternative to SDF and NaF varnish as in-office fluoride therapy.

The present study aimed to add to the limited research on the difference in remineralizing potential of these remineralizing agents in primary teeth by comparing the mineral content after their application. The promising results have implications for the arrest and management of early carious lesions in children.

One of the limitations of the present study was the difficulty to simulate the complicated intraoral conditions due to the regular change of pH. Also, the flow of saliva may alter the removal of varnishes from enamel. Although the antibacterial properties of silver compounds were well known, they were not the study's aim. It is also necessary to investigate the safety of NSF before its application in the clinic. Consequently, further studies are recommended in evaluating the oral factors, the antimicrobial potential and safety in clinical settings.

CONCLUSIONS

Based on the results of the present study, it can be concluded that:

- Nano silver fluoride is as effective as SDF and NaF varnish in remineralization of enamel caries like lesions in primary teeth. Moreover, nano silver fluoride showed a notable increase in F compared to SDF and NaF varnish.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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