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REMINERALIZING EFFECT OF SILVER NANOPARTICLES ADDED TO FLUORIDATED PIT AND FISSURE SEALANT ON CARIES LIKE LESIONS IN PERMANENT TEETH: IN VITRO STUDY

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ABSTRACT

BACKGROUND: The present study was designed to evaluate the effect of silver nanoparticles addition to fluoride-containing pits and fissure sealant (PFS) on the remineralization of enamel caries-like lesions in permanent teeth in comparison to fluoridated and non-fluoridated PFS.

MATERIALS AND METHODS: Fourty four sound human premolars with standardized occlusal window were immersed in the demineralizing solution for 96 hours to create initial carious lesions on the exposed enamel surface. They were randomly assigned into four groups according to the sealant used: GroupI: silver nanoparticles (AgNPs) added to fluoridated PFS Group II: fluoridated PFS Group III: non-fluoridated PFS Group IV: negative control (received no treatment). The specimens underwent pH cycling for 10 consecutive days. The enamel mineral content in weight % at baseline, after demineralization and pH cycling were evaluated quantitatively by Energy Dispersive Xray (EDX) and five teeth from each group were randomly selected for qualitative assessment of enamel sealant interface topography by Scanning Electron Microscope (SEM).

RESULTS: Post-treatment quantitative results revealed statistically significant higher mean Calcium (Ca), Phosphorous (P) content, and Ca/P ratio in the AgNPs group than the fluoridated PFS group (33.23 ± 1.09, 16.20 ± 0.51, and 2.03 ± 0.12 respectively) with no significant difference regarding the fluoride (F) content ($P<0.05$). However, the fluoridated PFS group showed higher mean Ca, P content, and Ca/P ratio as compared with the non-fluoridated and negative control groups (29.68 ± 1.31, 13.32 ± 0.93, and 1.95 ± 0.11 respectively). While the last two groups did not show any significant mineral gain. The qualitative finding revealed a white zone formed in both the AgNps and the Fluoride added PFS groups. However, no such zone was noticed in the Non-fluoridated PFS group.

CONCLUSION: AgNPs addition enhanced the remineralization potential of fluoridated PFS.

KEYWORDS: Silver nanoparticles, Fluoride, Sealants, Remineralization, Permanent teeth

RUNNING TITLE: Remineralizing effect of silver nanoparticles.

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INTRODUCTION

Dental caries is one of the predominant diseases worldwide with prevalence up to 60%- 90% in school-aged children in a few nations.(1) Tooth surfaces have significantly different susceptibility to caries. About 90% of caries in children's permanent teeth occur in pit and fissure alone as they are considered a complicated deep grooves within the enamel surface that are vulnerable to the deposition of food residues and bacteria.(2, 3)

As first described over 100 years ago, the underlying mechanism of dental decay is clear in concept.(4) Normally, the salivary pH is between 6.7 to 7.4,(5) but once the cariogenic bacteria break down carbohydrates, acids are released, causing a drop in the salivary critical pH below 5.5.(6) The formed acids result in the liberation of calcium (Ca) and phosphorus (P) minerals from surface and subsurface enamel. This would lead to the formation of white spot regions " early carious lesions".(7) These white spots are considered the first sign of demineralization that occur at the atomic level before it can be visually seen as a frank cavitation.(8)

The last decade witnessed a paradigm shift of the dental field from a curative to a primarily preventive one. The concept of minimally invasive treatment of dental caries, early detection, and early reversal of incipient carious lesions started to arise using a variety of remineralizing agents such as fluoride and calcium phosphate compounds.(9, 10) To date, fluoride is considered the gold standard remineralizing agent among many others still under research.(11) Remineralization allows for the redeposition of the lost mineral elements, mainly Ca and P, in the form of hydroxyapatite crystals in the voids of the demineralized enamel. In addition, Fluoride ions (F) presence in the oral environment will lead to fluorapatite crystals deposition, which are larger and more resistant to demineralization.(12, 13)

Pit and fissure sealants (PFS), a resin coating that binds micromechanically to the tooth surface, is widely known for the prevention of occlusal caries as well as the inhibition of non-cavitated occlusal carious lesions progression.(14, 15) Sealing occlusal pit and fissure can modify them into smooth surfaces, thus hindering the bacterial colonization and making it easier to clean.(16) However, PFS leakage is still a concern which may lead to bacterial invasion and secondary caries.(17) Therefore, there is an increasing need to improve remineralization potential of PFS in addition to its preventive goal.(18)

With the innovation of nanotechnology, new modalities have been proposed for dental caries management, such as AgNPs which started to have a promising use in the field of dentistry.(19) Several studies(20-23) have investigated the incorporation of AgNPs to various agents as mouthwashes, fluoride varnish, and resins for its bactericidal and bacteriostatic effect against streptococcus mutans the main causative organism of dental caries. However, its role in regulating the balance between the demineralization and remineralization process is underestimated.(24, 25)

Little is known about the remineralizing capability of AgNPs' addition on PFS; therefore, our study was designed for quantitative and qualitative assessment of silver nanoparticles addition to fluoride-containing pit and fissure sealant. The null hypothesis of this study was that there was no difference in the mineral content or in the surface topography of the enamel between different tested types of sealants.

MATERIALS AND METHODS

The research proposal was approved by the Ethical Committee of the faculty of Dentistry, Alexandria University, Egypt (IRB 00010556 – IORG 0008839). The sample size was estimated based on the results of Silva et al.,(26) where (mean ± SD) Ca content was 350 ± 65 when a fluoridated PFS was used, and 275 ± 40 for untreated enamel. According to Salas-López et al(27), addition of AgNps to PFS reduced tooth demineralization significantly and likely increased remineralization when compared to conventional sealant. The study power was 80% with 5% alpha error. Therefore, the study had greater power to detect difference between this group and the control group when all other factors are held constant,(28) Based on comparison of means, sample size was calculated using MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) to be 10 per group, increased to 11 to make up for laboratory processing errors.

Specimens' preparation

A total of fourty four human premolars freshly extracted for orthodontic purposes due to serial extraction were collected from the outpatient clinic of the Faculty of Dentistry and private dental clinics. The teeth chosen were sound, free from caries, cracks or developmental defects.(17) They were examined using magnifying lens to rule out the presence of cracks. The occlusal surfaces of the teeth were cleaned with fluoride free prophylaxis paste using rubber cups at low speed.

First quantitative analysis, the baseline enamel mineral content was assessed by Energy Dispersive Xray (EDX). The level of Ca, P, and F on the enamel surface was analyzed at 2 points which were standardized through all the experiment. One at the midpoint of the base of the fissure, while the other 2mm away from the 1^{st} point in the middle of the slope of the buccal cusp, signifying the future junction between the enamel surface and the PFS.

Caries like lesion formation

A double layer of an acid resistant nail varnish was placed on all the teeth surfaces except the occlusal surface.(29) Each tooth was placed in 15ml of demineralizing solution (2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide(KOH) at a pH of 4.4) for 96 hours according to the method applied by ten cates (1982)(30) to produce artificial enamel carious lesions. The teeth were then rinsed with deionized distilled water and placed in artificial saliva (500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to maintain a pH of 6.8).(31) A second quantitative assessment of the enamel mineral content by EDX was done as described before.

Preparation of the Test Sealant:

The entire content of one syringe (2.5ml) of TEETHMATE™ F-1 sealant (KURARAY CO., Umeda, Kita-ku, Osaka, Japan) was extracted and was then mixed with 6.45µg/mL of AgNPs of particle size 40-80 nm (US- Research-Nanomaterials, Inc.) in a dry capsule using an amalgamator (de GotzenS. R.l, Italy) for 10 seconds to obtain a homogenous mix and then reloaded to the original syringe. The mix was done in a dark room.(32)

Experimental groups

According to the sealant agents used, the sample was divided randomly by using permuted block technique into four groups. The chemical composition, manufacturer, and application procedure of the tested sealant are presented in table 1. All steps were done according to manufacturer's instructions.

pH challenge

Following sealant application, teeth in all the groups were subjected to the pH-cycling dynamic regimen for a duration of 10 consecutive days. The aim of pH-cycling was to simulate the drop in the pH that occur in the oral cavity every day.(33) The specimens were immersed for 6 hours in the demineralizing solution, rinsed with de-ionized water, and then immersed for 18 hours in the remineralizing solution (1.5mM of Calcium chloride ($CaCl_2$), 2.2 mM of Sodium dihydrogen phosphate (NaH_2 Po_4), and 0.15 M of Potassium chloride (KCl) to maintain the pH at 7.0).(30) Solutions were freshly prepared every 24 h to prevent their super saturation.

Remineralization assessment

After 10 days, specimens were removed and prepared for quantitative and Qualitative assessment). A diamond disk was used to cut the root portion. The crowns were then sectioned buccolingually through the middle of the occlusal surface to obtain mesial and distal halves and care was taken not to damage the filled sealant.(33)

Third quantitative assessment the mesial and the distal halves of each tooth of all the tested specimens were assessed by EDX at the same two points as described before and the mean values were taken.

Qualitative assessment five teeth from each group were randomly chosen. Each half was dehydrated by ascending grades of ethyl alcohol 50%, 70%,90%, and absolute alcohol. Then vacuumed and gold sputter-coated with the gold-palladium layer before examination by Scanning electron microscope SEM JSM-5300 at operating magnification x250 and x1000 at 20 kV to study the enamel-sealant interface topography. The presence of white zone at the interface was considered positive for remineralization.(17) Qualitative evaluation post-treatment was performed to confirm the quantitative analysis.

Statistical analysis

Normality was checked for all variables using descriptive statistics and normality tests. All variables showed normal distribution, so means and standard deviation (SD), and parametric tests were used. Comparisons of mean weight percentage of the enamel mineral content (Ca, P, and F) *between* the four study groups were done using one-way ANOVA, while comparisons of mean weight percentage of the enamel mineral content (between sound, demineralized and re-mineralized enamel) *within* each group were done using repeated measures ANOVA; both followed by multiple pairwise comparisons using Bonferroni adjusted significance levels. Significance was inferred at p value <0.05. Data were analyzed using IBM SPSS software for Windows (Version 23.0).

Table 1: EDX mean weight percentage of "Ca" in the four study groups at different time intervals

	Group I (AgNPs)	Group II (fluoridated PFS)	Group III (non-fluoridated PFS)	Group IV (negative control)	One way ANOVA (P value)
	Mean ± SD				
Sound enamel	33.65 ± 1.42	34.87 ± 1.39	33.40 ± 1.05	33.82 ± 1.13	2.38 (0.09)
Demineralized enamel	16.83 ± 2.93	15.73 ± 4.19	19.15 ± 1.27	18.54 ± 2.78	2.76 (0.056)
Re-mineralized enamel	33.23 ± 1.09 a	29.68 ± 1.31 b	20.14 ± 0.79 c	18.54 ± 2.78 c	167.41 (<0.001*)
Repeated measures ANOVA (P value)	143.80 (<0.001*)	143.80 (<0.001*)	607.28 (<0.001*)	400.54 (<0.001*)	
P value					
Sound – demineralized enamel	<0.001*	<0.001*	<0.001*	<0.001*	
Demineralized – re-mineralized enamel	<0.001*	<0.001*	0.04*	1.00	
Sound – re-mineralized enamel	0.19	<0.001*	<0.001*	<0.001*	

RESULTS

The results of the quantitative analysis (EDX) of the Ca, P content, and Ca/p ratio of the present study showed that there was no statistically significant difference between the four groups at the baseline and after the demineralization (P>0.05). While post treatment, the AgNPs group showed the highest significant Ca, P content and Ca/p ratio with mean value (33.23 ± 1.09, 16.20 ± 0.51, and 2.03 ± 0.12 respectively) with statistically significant difference with the other three groups (P<0.001). On the same line, the fluoridated PFS group showed statistically significant difference in the mean value of the Ca, P content and Ca/ P ratio (29.68 ± 1.31, 13.32 ± 0.93, and 1.95 ± 0.11 respectively) as compared to the non-fluoridated PFS group and the negative control group. Whereas, by comparing the non-fluoridated PFS group and the negative control group, the latter showed the least Ca, P content, and Ca/P ratio with a mean (18.54 ± 2.78, 10.77 ± 1.66, and 1.73 ± 0.16 respectively). However, there was no statistically significant difference detected neither in the Ca and the P content nor in the Ca/P ratio between both groups (P>0.05) (table 2, 3, and 4).

Within group comparison of the Ca content, results revealed that there was a statistically significant difference (P<0.001) in the three phases (sound, demineralized, and remineralized enamel) in the fluoridated PFS group and the non-fluoridated group. While the AgNPs group did not show a statistically significant difference between the sound and remineralized enamel (p=0.19). Moreover, the negative control group did not show a statistically significant difference between the demineralized and the remineralized enamel (p=1.00) (Table 2). Regarding the P content, within group comparison showed a statistically significant difference in the AgNPs group and the fluoridated PFS group. However, there was no statistically significant difference between the demineralized and the remineralized enamel in the non-fluoridated group and the negative control group (P=1.00) (Table 3). While within group comparison of the Ca/P ratio, results revealed that there was a statistically significant difference (P<0.001) in the three phases (sound, demineralized, and remineralized enamel) in the fluoridated PFS group. However, the non-fluoridated PFS group and the negative control group did not show a statistically significant difference between the demineralized and remineralized enamel (P=0.11, 1.00 respectively). Moreover, the AgNPs group did not show a statistically significant difference between the sound and remineralized enamel (p=0.48) (Table 4).

By comparing the F content between the study groups, results did not reveal a statistically significant difference at the baseline and after the demineralization in the four groups (p>0.05). While after remineralization, the AgNPs group showed the highest fluoride content (mean=1.86 ± 0.43) with a statistically significant difference with the non-fluoridated PFS group and the negative control group. However, there was no statistically significant difference with fluoridated PFS group (P>0.05). In the fluoridated PFS, there was a statistically significant difference detected in the mean value of F content (1.36 ± 0.37) when compared with the conventional non-fluoridated PFS and the negative control group (P<0.001). Although the conventional non-fluoridated PFS showed the least fluoride content with mean value (0.38 ± 0.24). There was no statistically significant difference detected with the negative control group (P>0.05) (Table 5).

Within group comparison of the F content, results showed that there was a statistically significant difference in the three phases (sound, demineralized, and remineralized enamel) in the fluoridated PFS group (P<0.001). While in the AgNPs group there was no statistically significant difference between the sound and remineralized enamel (p=0.21). Moreover, there was no statistically significant difference in the non-fluoridated PFS group and the negative control group between the demineralized and the remineralized enamel (P=0.47, P=1.00 respectively) (Table 3).

SEM observations

Regarding the qualitative assessment by SEM, examination was done at x250, and x1000 magnifications where the tooth enamel sealant interface was observed. The teeth in the AgNPs group showed an evident precise white zone at the tooth surface-sealant interface which appeared uniform (fig. 1A). The clear white zone appeared to be formed from the sealant towards the tooth structure (fig. 1B). Whereas the teeth in the fluoridated PFS group showed a relatively straight tooth surface-sealant along with a noticeable white zone uniformly formed along its border (fig. 2A, 2B). Although, the teeth in non-fluoridated group revealed an irregular tooth surface-sealant interface which showed some gaps (fig. 3A). There was no clear-cut white zone noticed (fig. 3B). While by the examination of the negative control group (untreated enamel), The demineralized surface appeared obviously irregular with evident surface roughness and scratchings (fig. 4A,4B).

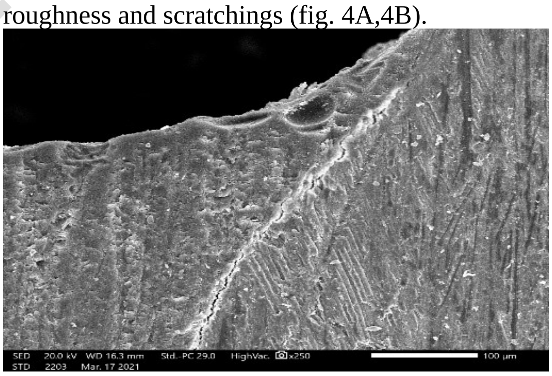

Figure (1A): Scanning electron micrograph of AgNPs group showing a clear white zone at the tooth surface-sealant interface. (Mag.x 250)

Figure (1B): Scanning electron micrograph of AgNPs group showing a straight, uniform line denoting the tooth surface-sealant interface. Note the definite white zone at the interface (white arrow) (Mag.x 1000)

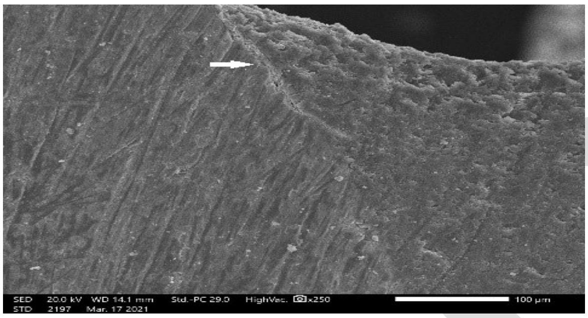

Figure (2A): Scanning electron micrograph of the fluoridated PFS group showing a visible white zone at the tooth surface-sealant interface (white arrow) (Mag.x 250).

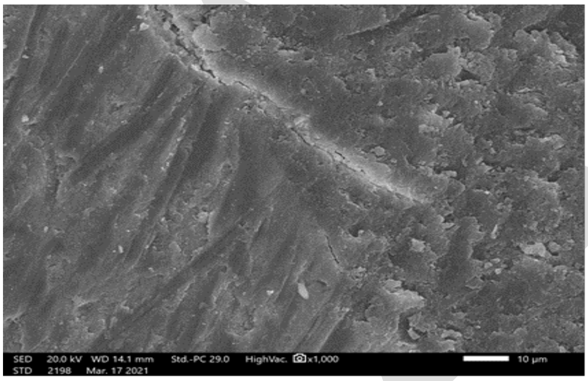

Figure (2B): Scanning electron micrograph of the fluoridated PFS group showing a remarkable white zone at the tooth surface-sealant interface which appeared relatively straight (Mag.x 1000).

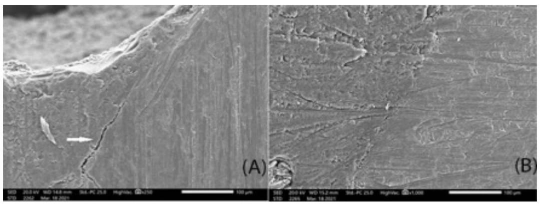

Figure 3 (A): Scanning electron micrograph of non-fluoridated PFS group showing an irregular tooth surface-sealant interface with an indistinct white zone (white arrow). Note the clear gap between the tooth and the sealant (Mag.x 250).

(B): Scanning electron micrograph of non-fluoridated PFS group showing no clear-cut white zone along the tooth surface-sealant interface (Mag.x 1000).

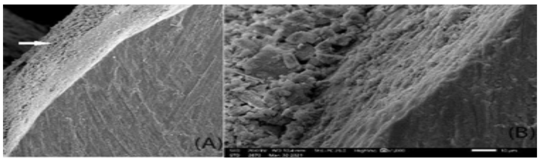

Figure 4 (A): Scanning electron micrograph of the negative control group showing an irregular demineralized enamel surface (white arrow). (Mag.x 250)

(B): Scanning electron micrograph of the negative control group showing evident surface roughness and scratchings of the demineralized enamel surface. (Mag.x 1000)

Table 2: EDX mean weight percentage of "P" in the four study groups at different time intervals

	Group I (AgNPs)	Group II (fluoridated PFS)	Group III (non-fluoridated PFS)	Group IV (negative control)	One way ANOVA (P value)
	Mean ± SD				
Sound enamel	16.41 ± 0.54	16.35 ± 0.82	15.55 ± 0.96	16.09 ± 0.95	2.20 (0.11)
Demineralized enamel	9.85 ± 1.52	10.27 ± 0.53	11.10 ± 0.97	10.77 ± 1.66	6.00 (0.14)
Re-mineralized enamel	16.20 ± 0.51 a	13.32 ± 0.93 b	11.22 ± 1.07 c	10.77 ± 1.66 c	48.90 (<0.001*)
Repeated measures ANOVA (P value)	171.00 (<0.001*)	169.12 (<0.001*)	149.10 (<0.001*)	58.79 (<0.001*)	
P value Sound – demineralized enamel Demineralized – re-mineralized enamel Sound – re-mineralized enamel	<0.001* <0.001* 0.02*	<0.001* <0.001* <0.001*	<0.001* 1.00 <0.001*	<0.001* 1.00 <0.001*	

Table 3: EDX mean weight percentage of "Ca/P" in the four study groups at different time intervals

	Group I (AgNPs)	Group II (fluoridated PFS)	Group III (non-fluoridated PFS)	Group IV (negative control)	One way ANOVA (P value)
	Mean ± SD				
Sound enamel	2.05 ± 0.13	2.13 ± 0.10	2.15 ± 0.10	2.11 ± 0.14	1.27 (0.30)
Demineralized enamel	1.71 ± 0.10	1.83 ± 0.25	1.73 ± 0.15	1.73 ± 0.16	1.76 (0.17)
Re-mineralized enamel	2.03 ± 0.12 a	1.95 ± 0.11b	1.81 ± 0.17 c	1.73 ± 0.16 c	25.12 (<0.001*)
Repeated measures ANOVA (P value)	2938.77 (<0.001*)	2189.32 (<0.001*)	1100.00 (<0.001*)	676.53 (<0.001*)	
P value					
Sound – demineralized enamel	<0.001*	<0.001*	<0.001*	<0.001*	
Demineralized – re-mineralized enamel	<0.001*	<0.001*	0.11	1.00	
Sound – re-mineralized enamel	0.48	0.03*	<0.001*	0.001*	

*Statistically significant at p value <0.05.
a,b,c different letters denote statistically significant differences between groups using Bonferroni adjusted significance levels

Table 4: EDX mean weight percentage of “F” in the four study groups at different time intervals

	Group I (AgNPs)	Group II (fluoridated PFS)	Group III (non-fluoridated PFS)	Group IV (negative control)	One way ANOVA (P value)
	Mean ± SD				
Sound enamel	2.23 ± 0.88	1.83 ± 0.32	1.82 ± 0.28	1.93 ± 0.67	1.05 (0.38)
Demineralized enamel	0.71 ± 0.56	0.62 ± 0.21	0.49 ± 0.27	0.56 ± 0.55	2.32 (0.09)
Re-mineralized enamel	1.86 ± 0.43 a	1.36 ± 0.37 a,b	0.41 ± 0.24 c	0.51 ± 0.32 c	23.58 (<0.001*)
Repeated measures ANOVA (P value)	22.79 (0.001*)	59.27 (<0.001*)	166.10 (<0.001*)	106.62 (<0.001*)	
P value					
Sound – demineralized enamel	0.003*	<0.001*	<0.001*	<0.001*	
Demineralized – re-mineralized enamel	<0.001*	0.001*	0.47	1.00	
Sound – re-mineralized enamel	0.21	<0.001*	<0.001*	0.001*	

*Statistically significant at p value <0.05.
a,b,c different letters denote statistically significant differences between groups using Bonferroni adjusted significance levels

DISCUSSION

Our study aimed to assess the remineralizing ability of AgNPs using an in-vitro remineralization model. Based on the findings of the present study, there was a variation in the remineralization ability observed between different tested types of sealants. Thus, the null hypothesis was rejected.

In the current study, quantitative elemental analysis was assessed for each specimen at three different phases: baseline, after demineralization, and after remineralization in each group using EDX which is a microanalytical technique to detect the weight percent of the mineral element Ca, P, and F from outer enamel surface.(34) The remineralization process depends mainly on the mineral changes that occur in the dental hard tissues structure. The level of Ca, P, and F elements in the enamel surface is considered an indication of the demineralization or the remineralization rates, and assessing these levels can denote the lesion progression. Therefore, this technique is helpful in comparing the effect of different sealant material on demineralized enamel surface.(35)

Our results revealed that the highest values of the mineral content were for the sound enamel. Whereas, after the immersion in the demineralizing solution for 96 hours, the values were notably decreased in all the four groups denoting mineral loss. While post-treatment, the AgNPs group and the Fluoridated PFS group showed a significant increase in the mineral content. Although, the first group showed the greatest Ca, P, and Ca/P values nearly to that of the sound enamel. There was an insignificant difference present regarding the F content as both are the same fluoridated sealant used except for the addition of silver nanoparticles in the first group. Therefore, it can be assumed that both sealants were able to remineralize the demineralized enamel. This result was consistent with aldhaian et al (36) who found that the fluoride and silver nanoparticles showed significant effect in the reduction of the mineral loss and the lesion depth in a dose response manner, indicating their effect in the demineralization prevention.

The remineralizing effect of F depends mainly on the F concentration in the saliva. In the presence of low fluoride ion concentration from fluoridated PFS, the Ca and P ions released from the enamel are reprecipitated as fluorapatite crystals instead of hydroxyapatite crystals. While on the other side, if the fluoride ion concentration is elevated, calcium fluoride (CaF_2) will be formed on the enamel surface instead of fluorapatite. This reaction is considered unfavorable as CaF_2 can be easily washed by water and saliva. However, the addition of heavy atoms such as silver atoms from the AgNPs added PFS on the enamel surface causes the weakening of the signal of CaF_2 formation. Thus, the signal of fluorapatite crystals' formation will be increased .(37) Furthermore, Silver nanoparticles have the ability to penetrate into the carious lesion, bind to hydroxyapatite crystals, and release silver ions. The released silver ions can also cause insoluble silver chloride to percipitate on the dental hard tissue, which in turn increases its mineral density.(38) Hence, it could be hypothesized that there is a synergistic effect present between the AgNPs and the F and that the silver particles can accentuate the effect of F in the enamel remineralization. Several studies support this explanation.(39-41) However, Aldhaian et al (36) failed to assess this synergistic effect.

Moreover, in the fluoridated PFS group there was an increase in the Ca, P, F content, and Ca/P ratio post-treatment when compared with the non-fluoridated and the negative control groups. This finding is in agreement with those obtained by Salar et al(42) who concluded that the fluoride-releasing resin PFS was able to increase the demineralization inhibition greater than conventional non-fluoridated PFS but less than Glass ionomer PFS. On the other hand, the current findings disagree with Prabhakar et al(43) who reported that the demineralization inhibition in enamel adjacent to fluoridated and non-fluoridated PFS was comparable. This disagreement may be due to the technique used was different from that of the present study.

While in the non-fluoridated PFS group and the negative control group, the enamel mineral content post-treatment showed no increase when compared with the values obtained after demineralization. This result could be due to the fact that both groups did not receive any treatment with remineralizing agent. Therefore, they were not able to revert the artificial caries process by remineralizing the demineralized enamel.

For further confirmation of the previous results, Qualitative analysis using (SEM) was performed. Examination of the tooth surface-sealant interface was done. In the present study, the SEM findings were consistent with the post-treatment quantitative changes. The specimens treated with AgNPs and fluoridated PFS showed a marked white zone when compared to specimens treated by non-fluoridated PFS. However, in the AgNPs group, the white zone was more prominent, uniform, and appeared to be formed from the sealant to the enamel surface. While in fluoridated PFS the tooth surface-sealant interface was relatively straight with a white zone formed along with it. In non-fluoridated PFS, irregular tooth surface-sealant interface was present and white zone was not evident along it. Whereas, in the negative control group, the enamel surface appeared to be irregular. This finding is in agreement with Choudhary et al (17) who stated that the presence of a white zone is considered a sign of remineralization. A similar white zone was also detected by Park et al(44) and Utenja et al(29).

To best of the present authors' knowledge, no other in-vitro studies assessed the quantitative and qualitative changes that occur by the addition of silver nanoparticles to PFS for the remineralization of artificial caries lesions.

One of the limitations of this study is that no Fourier Transform Infrared spectroscopy (FTIR) degree of conversion experiment has been conducted to confirm that the addition of the silver nanoparticles to the pit and fissure sealant did not affect the degree of conversion of the monomer system. Another possible limitation is that the pH-cycling model used did not entirely simulate the pH fluctuation that occur frequently in the oral environment. Moreover, the findings of an in vitro study could not be the same as those of an in vivo study as the effects of oral factors such as saliva and dental plaque on enamel remineralization was not taken into account. Although the antimicrobial properties of silver products are well established, that was not the purpose of this research. Additional in vivo and in vitro studies are therefore recommended to assess the oral factors effect and the antimicrobial properties of silver nanoparticles when added to PFS. Furthermore, conduction of micro CT analysis has been recommended for further studies.

CONCLUSIONS

The findings of the current study were promising as they showed an improvement in the mineral contents and the dental structure treated with AgNPs added PFS, indicating that it was not only able to prevent the progression of incipient enamel caries but was also able to revert the process by enhancing the remineralization.

Abbreviations

AgNPs: Silver Nanoparticles
Ca: Calcium
EDX: Energy Dispersive x-ray.
P: Phosphorous
PFS: Pit and Fissure Sealant
F: Fluoride
SEM: Scanning Electron Microscope

CONFLICT OF INTEREST

The authors deny any conflict of interests related to the current study.

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