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THE EFFECT OF ENAMEL PRETREATMENT WITH NANOSILVER FLUORIDE ON PIT AND FISSURE SEALANT IN PERMANENT TEETH (IN-VITRO STUDY)

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ABSTRACT

INTRODUCTION: Recently, Nanosilver fluoride (NSF) has been raised in the research field of caries prevention. Pit-and-fissure sealants could have an additional antibacterial and remineralizing benefit through enamel pretreatment by NSF.
OBJECTIVES: The aim of the study was to evaluate in-vitro the effect of NSF pretreatment on shear bond strength, marginal seal, and quantity of color change of pit and fissure sealant in comparison to sealant only in permanent teeth.
MATERIALS AND METHODS: Fifty-four freshly extracted permanent teeth were collected and randomly allocated into two groups: Group I: (n=27) NSF was applied to the enamel prior to sealant application. Group II: (n= 27) enamel was etched and sealed. Each group was further divided into three subgroups according to the test applied. (n= 9 teeth each). Subgroup A: Shear bond strength was measured using a Universal Test Machine. The deboned area was then examined under a stereomicroscope to assess the mode of failure. Subgroup B: Microleakage samples were examined under a stereomicroscope to assess the degree of microleakage. Subgroup C: Color Assessment: After a sealant application, any change in sealant color was evaluated using a spectrophotometer.
RESULTS: There was no statistically significant difference between both subgroups neither in shear bond strength nor microleakage. The comparison of the final color of sealants in both subgroups showed a higher color change in subgroup Ic with a significant difference (P-value =0.001).
CONCLUSION: Pretreatment with nanosilver fluoride prior to pit-and-fissure sealant application did not affect neither its shear bond strength nor its sealing ability. However, it altered the sealant color to an extent that exceeded the clinical acceptance levels.
KEYWORDS: Shear bond strength , Pit-and-fissure sealants, NSF, Microleakage, Spectrophotometer
RUNNING TITLE: Effect of NSF pretreatment on pit and fissure sealant.

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INTRODUCTION

Pit and fissure caries is one of the most problematic dental lesions. The complex morphological shape of fissures is a prosperous place for bacterial growth, away from self-cleansing and oral hygiene measures. Also, the enamel rods distribution facilitates the transfer of infection to dental pulp rapidly (1). Consequently, pit and fissure caries accounts for 80 to 90 percent of total caries presents in permanent teeth .(2). Unfortunately, the caries onset in the first permanent molars occurs soon after their eruption.They are the highest caries risk followed by the second molars (3). The unawareness of the parents of the chronological age of teeth eruption and the difficulties to reach the dental clinic are the main causes to aggravate the condition (4).
A prospective clinical trial performed by Songur et al, (2019) (5) declared that the treatment of early childhood caries under General anesthesia or in the clinic didn't decrease the chance of carious infection of the permanent molars, justified by the colonization of species of bacteria in the oral cavity.
In fact, early prevention is quite important to ensure a good quality of life for children. Among the most famous minimally invasive protocols, dental sealants and topical fluoride application are the two most important caries preventive strategies (6).
Dental sealant is a flowable material that could infiltrate into the deep dental fissures, block the colonization of bacteria, and prevent the stagnation of food debris (3). The key problem of a dental sealant is the microleakage that occurred due to the polymerization shrinkage. This might facilitate the formation of gaps between the material and tooth structure (7). For this reason, pit and fissure sealants need reassessment every six months to avoid the development of dental caries. It

is important particularly in patients with a high risk of developing caries and insufficient oral health behaviors (8).
Topical fluoride agents are known for their capacity to fight dental decay (6). Fluoride varnish preparation is commonly used as a preventive care routine in dental clinics, although it is considered as a costly treatment, technique sensitive and needs to be applied at least twice yearly to be effective (6, 9). Therefore, this method does not appear to totally inhibit caries development (10).
As a consequence, combination therapy has been proposed. Fluoride was used as pretreatment of the enamel surface before sealant application. But, according to Frazer et al. (2017) (11) the fluoride varnish applied just before sealant placement, negatively affects the retention of the sealant.
Therefore, alternative preparations that provide further benefits may be a source of interest (12). Silver Diamine Fluoride (SDF) is simple, inexpensive, and more effective than fluoride varnish. This biomaterial combines the remineralization effect of fluoride and the antibacterial effect of silver. The synergism between them is the main cause of its predomination as an anti-caries agent in deciduous and permanent teeth (12). Furthermore, SDF solution showed greater success when applied under resin restorations without compromising their mechanical or adhesion properties but, it recesses the esthetic feature of the material. The black staining caused by SDF may be misdiagnosed as caries and give poor aesthetic for dental restoration. Also, it stains the oral mucosa and may cause ulceration (13,14).
Nanotechnology has become a key research field that brought beneficial application in medicine and healthcare (15). It contributed to overcoming SDF's disadvantages by reducing the silver particles into nanoscale which presents better remineralization and antimicrobial properties. Recently, Nanosilver fluoride (NSF) was introduced as a new experimental formulation that contains nano silver particles, Chitosan and Fluoride. NSF is a yellow solution that proved to be stable for 3 years and is ecofriendly (16). Chitosan is a natural biopolymer and positively charged. It has a protective and stabilizing ability as it can be adsorbed on the surface of silver nanoparticles. Wei et al. (17) declared that the antimicrobial and low toxicity properties of silver could be ameliorated by the combination with chitosan. Besides, its antimicrobial effect, they also assured its remineralization ability. It acts as a carrier agent and helps the material to penetrate easily into the demineralized enamel (18).
The nano agent has a distinct penetration ability that prevents the reaction of silver with the surrounding oxygen, which helps prevent staining the dental tissue into black (16). Moreover, NSF does not taste metallic and has low toxicity than SDF. The preventive mechanism of NSF was contributed by Nanosilver ions which prevents bacterial DNA replication and adherence to the enamel surface. It also inhibits the formation of Streptococcus mutants and lactobacillus (19). Also, NSF has a higher mineral release compared to other remineralizing agents (20, 21).
Despite the abundance of literature that shows the effectiveness of NSF as a preventive agent, scarce information is available on the effect of its use to treat the fissures before sealant application in terms of retention as measured by shear bond strength and sealing ability as depicted by microleakage. This triggered the interest to conduct further research. The proposed null hypothesis of the present study would be that fissures treated by NSF prior to sealant application show similar shear bond strength , microleakage, and esthetic properties as those protected by sealant alone.

MATERIAL AND METHODS

The research was performed at the Pediatric Dentistry and Dental Public Health, Dental Biomaterials Departments and Conservative Department, Faculty of Dentistry and Pharmaceuticals Department Faculty of Pharmacy, Alexandria University, Egypt. This study was approved by the research ethics committee (0054-08/2019) at the Faculty of Dentistry, Alexandria University.
The sample size was estimated based on the following assumptions: confidence level= 95%, study power= 80%, means shear bond strength of self-etch adhesive system pretreated with nano silver particles was 18.08±3.28 while it was 14.34±1.75 in self-etch adhesive system without nanosilver particles (control group) (22). Using these assumptions, the required sample size was calculated using G power 3.0.10 to be 9 teeth in each of the 6 groups with total sample of 54 teeth. Sample size was calculated using the G power 3.0.10-calculator tool. Sample size was calculated using the G power 3.0.10-calculator tool.
Fifty-Four freshly extracted human permanent molars and premolars (20 molars,34 premolars) were collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University Public Hospital. The teeth included were freshly extracted without caries, previous filling with no developmental anomalies or cracks. Teeth fulfilling the inclusion criteria were randomly allocated to one of two groups using a computer-generated random number.
Teeth were cleaned with fluoride-free prophylaxis paste using brushes at low speed and stored in distilled water until use. Teeth were randomly divided into two groups. Group I: (study group) (n = 27) for NSF treated fissures, followed by sealant application and group II: (n = 27) for sealant only. Each group was divided into three subgroups (n=9) to perform the tests (Figure 1). The sealant used was (Fisseal, Promedica, Germany).

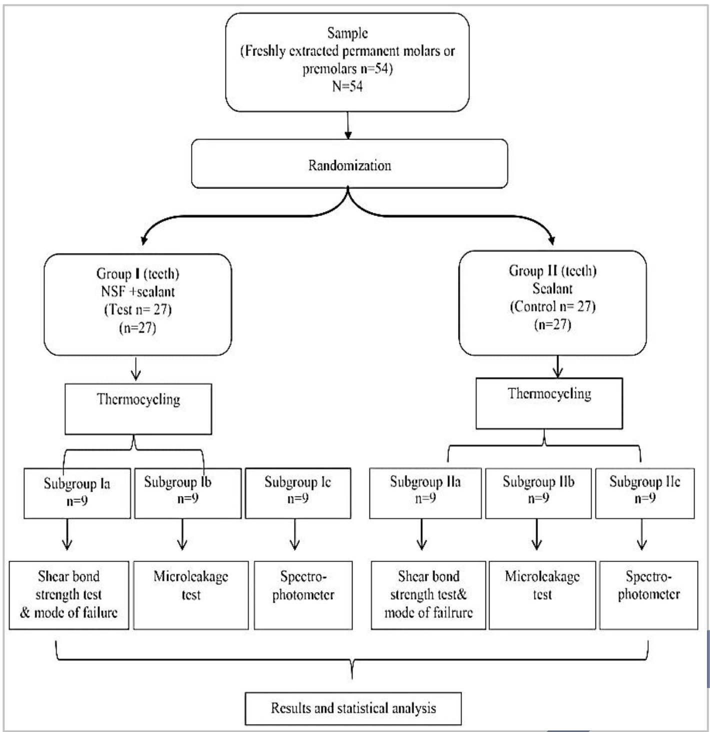

Figure 1: Flow chart

NSF preparation

According to Wei et al. (17) preparation of NSF solution was performed by extracting the silver nanoparticles via the chemical reduction of silver nitrate (1 mL, 0.11 M) with sodium borohydride (0.3 mL, 0.8 M) and chitosan biopolymer (28.7 mL, 2.5 mg/mL) as a stabilizing agent. Sodium fluoride (10,147 ppm of fluorine) was incorporated at the end of the experiment.

Shear Bond Strength Test

Teeth were mounted individually on acrylic blocks with the buccal surface perpendicular to the long axis of the block. The buccal surfaces were ground flat by 800 and 1000 grit wet silicon carbide and finishing paper under water cooling.

In subgroup Ia: teeth received pretreatment with NSF. Each tooth received 2 drops of NSF solution using a micro brush. The solution was left in contact with the tooth surface for 2 minutes then rinsed with a flow of deionized water (16). Universal bond (3M, ESPE, Adper™ Single bond universal Adhesive) was applied, dried for 5 seconds and light-cured for 10 seconds then the sealant (PROMEDICA Fisseal. Domagkstr, Neumunster Germany) was applied and cured using LED light-curing system (Ivoclar Vivadent, Germany)

In subgroup IIa: teeth were etched and sealed without pretreatment with NSF. The sealant was light-cured for 30 seconds according to the manufacturer's instructions.

The sealant was applied to the flattened buccal surface according to the manufacturer's instruction, using a readymade cylindrical plastic mold (4 mm in diameter and 3 mm in height). The plastic mold was placed perpendicular and centralized over the enamel surface and stabilized by sticky wax. The sealant was applied and cured. The plastic molds then removed carefully.

Teeth were thermocycled in a thermocycling apparatus for 500 thermal cycles, with altering the temperature between 5°±C and 55±5°C in a water bath with a dwell time of 30 seconds (23). Teeth were then immersed in saline for 24 hours. The teeth were mounted for assessment in the Universal Test Machine (Instron, Comten Industries, USA). The force was applied with a metallic loading blade placed as close as possible and parallel to the junction of the tested material and the enamel surface with a crosshead speed of 1mm/min. Shear bond strength was then measured by determining the force required to dislodge the sealant from the enamel surface. Bond strength was calculated in Megapascals (MPa) by dividing the load at failure (Newtons) by the adhesive surface area of the attachment (mm^2). The fracture assemblies for each tooth were evaluated by a single operator under a stereomicroscope (Olympus Co. Germany) at 20x magnification to determine the location and type of failure according to Peutzfeldt and Nielsen (24) as: cohesive failure (failure occurred within the substrate enamel or sealing material), adhesive failure (between sealing material and enamel) and mixed failure (adhesive and cohesive failures occurred simultaneously).

Microleakage test

The root apices of the teeth (13 premolars and 5 molars) were set in place using sticky wax. Each tooth was mounted in self-curing acrylic resin using copper cylinder molds with the occlusal surface facing upwards and perpendicular to the long axis of the block.

In subgroup Ib: fissures were pretreated with NSF as mentioned. Then they were etched and sealed. For group IIb: conventional sealant was applied in fissures only. All teeth in both subgroups were thermocycled as mentioned.

All teeth surfaces were polished with two layers of nail varnish except for 1.5mm of the sealant margins. Teeth were then immersed in a 1% solution of methylene blue dye for 24 hours. Teeth were rinsed with distilled water, dried, and sectioned longitudinally in a bucco-lingual direction. The degree of dye penetration at sealant/enamel interface was assessed using a light stereomicroscope (OLYMPUS SZ II. Olympus optical Co. Tokyo, Japan) under x60 magnification.

Dye penetration at the material/tooth interface of the enamel was scored as follows (25):

0= no evidence of dye penetration;

1= dye penetration of less than 1/3 from the margin of restoration;

2= dye penetration of more than 1/3 and less than 2/3 from the margin of restoration;

3= dye penetration of more than 2/3 from the margin of restoration.

Color assessment

In order to assess discoloration effect of the NSF on the sealant, a spectrophotometer was used to detect the color difference between subgroup Ic (test) and subgroup IIc (control). Quantitative color measurement was executed using the Commission International de I'Eclairage's (CIE Lab) uses three color coordinates L*a*, b*, The L* refers to the lightness coordinate, and its value ranges from 0 for perfect black to 100 for perfect white. The a* and b* are the chromaticity coordinates in the red–green axis and yellow–blue axis, respectively.The color difference corresponds to Delta E. Each tooth was mounted individually on acrylic blocks with the buccal surface perpendicular to the long axis of the block (26). The teeth were inserted in transparent blocks to eliminate the scattered light that could interfere with the result obtained during the spectrophotometer evaluation. The middle of the buccal surface of teeth met the round tip of the spectrophotometer in the same area each time to ensure the standardization of the measurement.

All the measurements were made on wet enamel surfaces to avoid alterations of enamel color caused by dehydration as recommended by other spectrophotometric studies (26). In subgroup Ic: 9 teeth received pretreatment with NSF, etched, then sealed, and in subgroup IIc: 9 teeth were sealed without pretreatment with NSF.

Color measurement was performed by positioning the flat surface of the portable spectrophotometer (VITA Easyshade Compact,Germany) against the center of the flat surface of the specimen. The assessment of tooth color in the 2 subgroups Ic and IIc was performed prior to the placement of the materials (as a baseline) and after sealant placement. Every reading was repeated three times for each specimen, and the average value over the three measurements was calculated (27). All data were collected, tabulated from each group and the mean values of each group were considered. The color difference of each group was mathematically calculated as ΔE values according to the following formula (27) $\Delta E^{*}ab = [(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}]^{1/2}$. The results were evaluated according to the clinical acceptance range (1.0<ΔE≤ 3.3) (28).

The baseline readings "T0" were done and repeated three times for each tooth before the test then the mean values of (ΔL*, Δa*, and Δb*) were calculated for each specimen. The readings "T1" were taken after sealant application in both groups and the mean values were calculated too. The measurements were taken and recorded by a single operator.

The color difference (ΔE) (T1-T0) values were calculated for each tooth according to the formula: **ΔE = [(ΔL*)2 + (Δa*)2 + (Δb*)2] 1/2.**

Intragroup *(i.e: ΔE before and after for each specimen)* and Intergroup (*i.e: Δ* E_t *of teeth with NSF and* ΔE_s *of teeth with sealants only)* then, the comparisons of the color changes (ΔE) were done between the 2 subgroups. *(All* ΔE_t *of the test were compared to* ΔE_s *of sealant)*

Statistical Analysis

Normality was checked for using descriptive statistics, plots (histogram and box plot), and Shapiro Wilk test indicating normality of Shear bond strength and ΔE. Shear bond strength and ΔE were presented using mean (SD) and 95% CI. Mode of failure and different levels of microleakage were displayed using count and percent.

Independent t-test was applied to assess differences in Shear bond strength and ΔE. Mann Whitney U test was applied to compare between groups regarding microleakage scores. Monte Carlo simulation method for Pearson's chi-square was used to assess differences in mode of failure and different levels of microleakage. Significance level was set at p-value ≤0.05. Data were analyzed using IBM SPSS statistical software (version 25).

RESULTS

Shear bond strength test

The mean± SD values of the shear bond strength were 40.44 ±12.07 and 39.2 ±14.3 in subgroups Ia and IIa respectively. Shear bond strength evaluation showed that there was no statistically significant difference between the 2 subgroups. (P=0.847, Table1).

Regarding mode of failure, Pearson chi-square test showed that in both subgroups 0% had cohesive mode of failure and 44.4% had mixed mode of failure. 55.6 % had adhesive mode of failure in both groups. There was no statistically significant difference in the distribution of modes of failure between the two subgroups where P=1.00.

Microleakage

Different levels of microleakage were displayed using count and percent. The percent of microleakage was 55.5% and 66.6% in subgroups Ib and IIb respectively (Table 2) with no significant difference between groups where P=0.625

Color assessment test

The color difference was mathematically calculated according to Δ E equation and compared to the clinical acceptance range (1.0≤ ΔE≤3.3). All Δ E in both subgroups were more than the 3.7.

The mean of all ΔE values were 18.82 and 13.1 in subgroups Ic and IIc respectively. Using independent t-test, the comparison between both subgroups showed that there was a significant difference between the subgroups (P=0.0001, Table 3)

Table 1: Comparison of mean (SD) shear bond strength (SBS) between Ia subgroup and IIa subgroup

SBS	Subgroup Ia(n=9)	Subgroup IIa(n=9)
Mean (SD)	40.44 (12.07)	39.22 (14.32)
95% CI	31.16, 49.73	28.22, 50.23
T test	0.196	
***P* value**	0.847	

Table 2: Comparison of microleakage percentage between Ib and IIb subgroups

Microleakage	SubgroupIb(n=9)	SubgroupnIIb(n=9)
	n (%)	
Score 0	4 (44.4%)	3 (33.3%)
Score 1	3 (33.3%)	3 (33.3%)
Score 2	2 (22.2%)	1 (11.1%)
Score 3	0 (0%)	2 (22.2%)
X^2	2.476	
***P* value**	0.625	

Table 3: Comparison of ΔE between subgroup Ic and subgroup IIc

SBS	Subgroup Ic (n=9)	Subgroup IIc (n=9)
Mean(SD)	18.82 (3.04)	13.10 (1.29)
95% CI	16.49, 21.16	13.01, 14.99
T test	4.388	
***P* value**	0.0001*	

*Statistically significant at *p* value ≤0.05

DISCUSSION

The trendy approach of Minimal Intervention Dentistry (MID) first relies on caries prevention and non-invasive management of early carious lesions. Pit-and-fissure sealants have become one of the major elements of the comprehensive caries management strategy. Recently, a new generation of bioactive dental materials has evolved with the antibacterial or remineralizing supplementary property. This could be achieved through the combination of therapeutic materials. The nanoparticle materials have been innovated to accomplish these objectives. Therefore, the pretreatment with NSF can increase the protective effect of pits and fissure

sealants against the formation of carious lesions with an additive advantage of omitting tooth staining.
Permanent teeth have narrow and deep pits and fissures with complex morphology that allow them to be more susceptible to plaque retention and inaccessible to mechanical cleaning.
In the present study, Fisseal™ pit-and-fissure sealant was used. It is a light cured, fluoride-releasing, unfilled, resin-based sealant. According to Hassan et al. (28) who evaluated the clinical retention of different types of sealants after 3,6,9,12 months, it was found that Fisseal as unfilled resin sealant proved to be highly retentive along all intervals. Fisseal is white opaque sealant which is different than the enamel color. It is faster and easier to be seen during application and during reassessment of sealant retention over long time periods than the clear sealant. The importance of color assessment is not just for verifying good esthetics but also, for evaluating proper function of restoration.(29)
When fissures were pretreated with NSF prior to sealant application, only two drops were added (20). Excessive use of NSF can affect the mechanical properties of sealant. The rinsing step is done to refresh the surface and to remove the excess without affecting the infiltrated material. Also, according to Lutgen et al. (30) the washing step improves the bond strength with the adhesives.
The universal bond was applied before the sealant application. Following an evidence-based in 2008 from the American Dental Association and the American Academy of Pediatric Dentistry reported that the use of adhesive systems before sealant application had better influence on sealant retention (3). The universal bond was used as its monomers composition differs from other adhesive systems. The self-etching option allows good chemical and micromechanical bonding to the enamel surface and it can alternate the conventional etching (31,32). Moreover, Universal adhesives are simpler, more user-friendly, and less technique-sensitive, and reduce patient chair time. These privileges are advantageous in pediatric dentistry (32).
Regarding the results of the shear bond strength test, both groups showed comparable values and mode of failure with no statistically significant difference P= (0.847). The results revealed that NSF pretreatment did not disturb the retention of adhesive and sealant used. This out rules the assumption that pretreatment with NSF can minimize the adhesion properties of the restoration.
Considering that NSF is a modification of SDF and due to lack of evidence on NSF pretreatment, the results were compared with the SDF studies. The results of the present study were in agreement with Pérez-Hernández et al. (14) who assessed the effect of pretreatment of SDF on the sealant adherence in permanent molars. They found that the SDF application didn't disturb the retention of the sealant. And the result was statically not significant. (P=0.801)
Wu et al. (33) evaluated the microtensile bond strength of composite restoration on dentin treated by SDF of primary molars, they found that the compatibility and the retention might be stronger in the presence of SDF.
A limitation of the study was that shear bond strength testing requires a flattened tooth surface with ground and polished enamel. This method of specimen preparation is different from that of the clinical condition where sealants are applied to irregular pits and fissures which provide more mechanical retention. Consequently, clinical testing would overcome this limitation of the SBS study design.
The results showed a decrease in microleakage in the fissures pretreated with NSF but, the difference was not statistically significant (P=0.457)
The previous results may be regarded as coinciding with those of Pérez-Hernández et al. (14) who assessed the effect of pretreatment of SDF on sealant microleakage. They reported that a tooth surface treated with silver diamine fluoride before the application of fissure sealants decreases the microleakage of the pit and fissure sealant, but the results did not score a significant difference. This also agreed with Elhabashy and El Tekeya (34).
In the NSF group, the presence of a white precipitate was observed on the enamel surface. This observation was also reported by Elhabashy and El Tekeya (34) Additionally, Nozari et al. (19) stated that NSF has a greater tendency to react and form a protecting layer to strengthen the dental tissue against the attack of demineralizing agents.
Ata et al. (35) and Abo El Soud et al. (36) mentioned that this precipitate may be due to the high diffusion of the small silver nanoparticles that allow more deposition of minerals like calcium and phosphorus. Unlike silver, which is a heavy metal deposit that prevents minerals to be precipitated. Furthermore, as stated by Soekento et al. (37) NSF has the higher fluoride ion release compared with SDF. This may boost the formation of white calcium fluoride (CaF) precipitate and increase the microhardness of the NSF treated enamel. Moreover, according to the result of the present study, it is an ideal pretreatment of enamel without compromising the microleakage of dental sealant.
The Vita Easy shade spectrophotometer was selected to quantitatively record the enamel color changes that occur due to sealant application. It has higher reliability and accuracy compared to other devices like digital photography (38).
The results showed that there was a significant difference between the initial enamel color and its color after application of the sealants in both groups, i.e., ΔE exceeded the critical value of clinical detection (3.7 units) and was clinically visible. Results showed that the white color of the sealant was different from the enamel color. Studies assessed the variation in tooth color either bleaching or staining potential by measuring ΔE and. The luminosity (L*) was the color axis of the CIELAB system that clarifies the variation between white and black. The higher ΔE values indicated more difference in color (38).
The comparison between the final color of sealants (ΔE) in both subgroups showed a significant difference as, $P < 0.05$. A yellow halo was observed around the sealants in the NSF group. This yellow color of the solution may remain even after washing of the material due to the precipitation of silver ions. This may be caused by the reduction of Ag^+ during its preparation which changed the solution from colorless to light yellow. It was also suggested that the chitosan present in the solution causes agglomeration and adherence to the dental surface and is responsible for the formation of this yellow layer (39).
This outcome matched the result of the in vitro study of Espíndola-Castro et al. (40) that compared the dentin staining after NSF and SDF application. They calculated the difference in baseline color with the ΔE equation. They found that the color difference from NSF was 18.59 and from SDF was 49.22. This means NSF causes yellow staining and SDF

causes a black pigment. They confirmed also, that this undesired yellow staining of NSF was easily removed after a small brushing cycle (3 minutes) or even by rubbing a gauze. By then, the ΔE values returned to the baseline (40).

CONCLUSION

The pretreatment with Nanosilver fluoride to pit-and-fissure sealant did not affect either its sealing ability or retention.It altered the sealant color to an extent that exceeded the clinical acceptance levels.It slightly affects the color of the sealant to an acceptable extent.

CONFLICT OF INTEREST

The authors declare that they have no conflicting of interests

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