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EFFECT OF DIFFERENT ETCHING TIMES ON SEALANT PROPERTIES APPLIED TO FLUORIDE TREATED TEETH: AN IN VITRO STUDY

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ABSTRACT

This study aimed to investigate the effect of fluoride varnish on microleakage and the interfacial micromorphology of pit and fissure nano sealant after different etching times.

Materials and methods: One hundred freshly extracted third molars were used in this study. They were randomly allocated into control group (20 teeth) and test group (80 teeth) which was further subdivided into 4 subgroups each one contained (20 teeth) according to the etching time. Teeth of the test group were subjected to fluoride varnish application. The next day, each subgroup was acid etched according to its specific time (20, 40, 60, and 120 seconds for groups I, II, III and IV respectively). Nano fissure sealant was then applied to all teeth according to the manufacturer instructions. Teeth in the control group received the nano sealant without fluoride application. Ten teeth from each subgroup were used for interfacial micromorphology scanning, and the other 10 teeth were used for microleakage evaluation.

Results: The scanning electron microscopic examination revealed that the formed tags varied between the groups. The shortest tags were found in subgroup I (between 2.24-3.22 μm), while the largest were in subgroup IV (35.19- 35.24 **μm**). Score 0 microleakage was the significantly highest one in all groups. The comparison between test groups and control group revealed significant differences in score 1 and 3 only.

Conclusion: Application of fluoride did not affect the sealant application especially for the etching times 40 and 60 seconds.

KEY WORDS: Fluoride varnish – Nano sealant – Etching time.

INTRODUCTION

Caries prevalence has been reduced in most industrialized countries. However, dental caries is still a problem in some populations. It was reported to have increased prevalence in some developing countries[1,2].In Egypt, caries prevalence was measured at different governorates since 1962. About 81.4% of the 6-19 years studied population had caries[3]. In a more recent study, this percentage did not differ a lot as 77% of the examined 12 years old children in Demietta governorate had caries[4].

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But later on, this percentage much decreased as it was (40.9%, and 39.4% for female and male subjects of the same age respectively) in Mansoura city at 2012 [5].

The majority of the detected caries among children and adolescents was reported to affect the pit and fissure surfaces of first molars[6]. This caries pattern advocates the utilization of some preventive measures like fissure sealants and topical fluoride application.

Dental sealants are resin materials that are used to physically seal deep pits and fissures preventing the accumulation of plaque and food debris in such areas, thus reducing caries. The success of the sealants depends on the proper enamel etching [7].

Fluoride is one of the most commonly used caries preventive measures. It is reported to reduce caries and plaque accumulation both in children and adults[8,9]. In 2001, Whelton recommended the combined use of fluoride and sealants as caries preventive measures[10].

Although fluoride is reported to reduce the enamel solubility in acidic conditions, its effect on resin or sealant attachment is still argued. Wang and Sheen[11] reported that fluoride application before acid etching has no effect, while, Meng et al[12] reported that it influences the enamel etching prior to resin application. Later on, the application of fluoride varnish was reported to have no effect on the shear bond strength of orthodontic brackets to enamel[13]. In another study, it was concluded that the proper sealant retention is obtained when the acid etching step was done two weeks after fluoride application. Moreover, to apply the sealant before two weeks, the etching time should be increased [14].

Several studies recommended that fluoridated enamel should be etched for longer times that ranged from 75 – 180 seconds instead of the conventional time of 15-20 seconds[15,16]. These results were supported by the scanning electron microscope and enamel topography changes studies[17].

There is a controversy about the effect of fluoride on the retention of pit and fissure sealant, so this study aimed to investigate the effect of fluoride varnish on microleakage and the interfacial micromorphology of pit and fissure nano sealant after different etching times.

MATERIALS AND METHODS

100 sound permanent molars extracted due to impaction were selected. The teeth were cleaned and disinfected in 0.5% chloramines solution for 24h, then stored in normal saline at 37˚ till the time of use. Prophylaxis was done for all teeth using pumice and brush at low speed. Excess of pumice powder was then removed by moving fine explorer on pits and fissures' base. Teeth were randomly allocated into two main experimental groups; test group (received fluoride varnish before sealant application) and control group (sealant was applied without fluoride). In the test group (80 teeth) the etching was done one day after fluoride application. The teeth were divided into 4 subgroups (20 teeth each) according to the etching time as follows; subgroups I, II, III, and IV where the enamel was etched for 20, 40, 60, and 120 seconds respectively. In control group (20 teeth) sealant was applied without fluoride application according to the manufacturer instructions[18]. Ten teeth from each subgroup were used for scanning electron microscope and the other ten teeth were used for microleakage testing.

Technique of application

Fluoride varnish (Duraphat varnish, 5% NaF=2.26%F, Colgate-Palmolive, Germany) was applied using paint on technique[19]. After dryness of the varnish the teeth were stored in artificial saliva to the next day. Teeth were air dried and subjected to sealant application according to their groups. All teeth were acid etched by phosphoric acid 37% (H3PO4, FineEtch 37, Spident Inc., Korea). Nanofilled resin based fissure sealant (GrandioSeal, Voco, Cuxhaven, Germany) was then applied to the teeth according to the manufacturer instructions. All

teeth were stored in artificial saliva till scanning and microleakage tests were performed.

Scanning electron microscope

Interfacial micromorphology evaluation

Sectioned teeth were examined by scanning electron microscope (SEM). The sections were polished with a 600-grit abrasive paper. The polished surfaces were etched for 20 seconds with 37% phosphoric acid to remove the smear layer followed by deproteinization in 5% sodium hypochlorite for 5 min. after being rinsed with distilled water, each sample was mounted on stubs, sputter coated with gold and examined with SEM[20].

Microleakage

In preparation for **dye penetration (leakage)** test, the crowns were coated with two layers of nail polish interposed by a layer of wax, leaving exposed a 1.5-mm window around the sealant margins and immersed in 2% buffered methylene blue dye for 24 h. Thereafter, the nail varnish and wax were removed with a sharp instrument. After that the teeth/resin blocks were sectioned longitudinally in buccolingual direction with a water-cooled slow speed operated diamond coated disc (Buehler, USA) thus providing 2 sections per tooth. The sections were examined with USB digital microscope (Scope Capture Digital Microscope, Guangdong, China) at 65x magnification and photographed using image analysis software (Image-J 1.45K, National Institute of Health, USA) and a ranked scale was used to score dye penetration as follows:[21, 22].

0= no dye penetration;

1= dye penetration limited to the outer half of the sealant;

2= dye penetration extending to the inner half of the sealant;

3= dye penetration extending to the underlying fissure.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS), software for Windows version 17. Data from microleakage scores were collected and expressed as numbers and percentages. Chi-square test was used for statistical analysis and the significance level was considered at 5%.

RESULTS

Electro-Microscopic results

The scanning electron microscopic examination revealed that the sealant in all groups was in close contact with the enamel surface. However, the formed tags varied between the groups. They were very short in subgroup I and accordingly the sealant penetrated these tags to a short distance that ranged between 2.24-3.22 μm. For subgroup II, the scanning results showed that when enamel was etched for 40 seconds, the formed enamel tags ranged between 7.24- 10.24 ***μm***. In subgroup III, the formed enamel tags were 25.47- 34.14 ***μm. On the other hand, the formed enamel tags in*** subgroup IV ranged between 35.19- 35.24 ***μm.***

Microleakage results

Table (1) showed that score 0 was the significantly highest one in all groups, while score 2 was not found in any group. Score 1 was not found in control group. However score 3 was found in control group and subgroups I and II. Significant differences were found in each subgroup between different scores. The comparison between test groups and control group revealed significant differences in score 1 and 3 only.

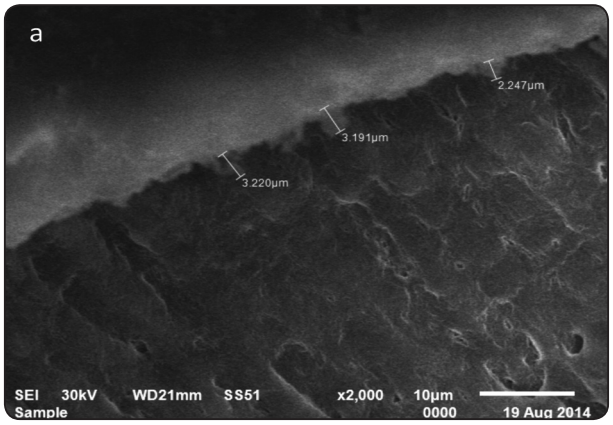

Fig. (a) Micrograph of a sample in subgroup I showing that sealant enters the very short enamel tags.

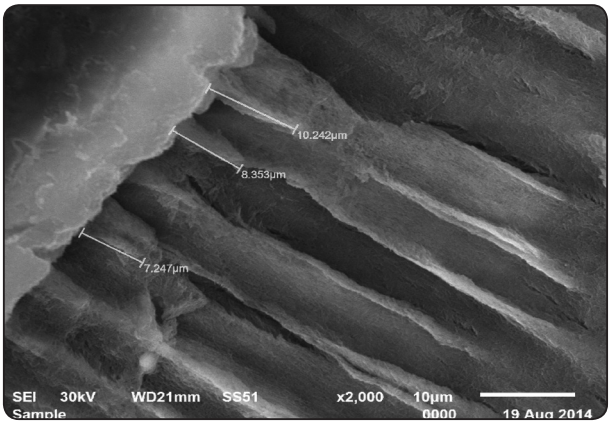

Fig. (b) Micrograph of a sample in subgroup showing that the sealant enters more elongated enamel pores.

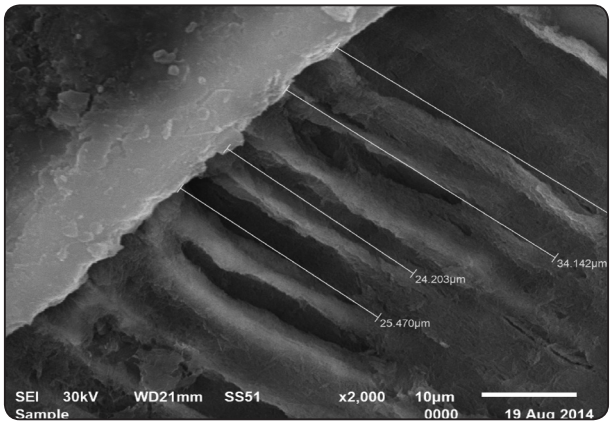

Fig. (c) Micrograph of a sample in subgroup III showing that sealant enters the enamel pores that ranged from 25.47-34.14 μm.

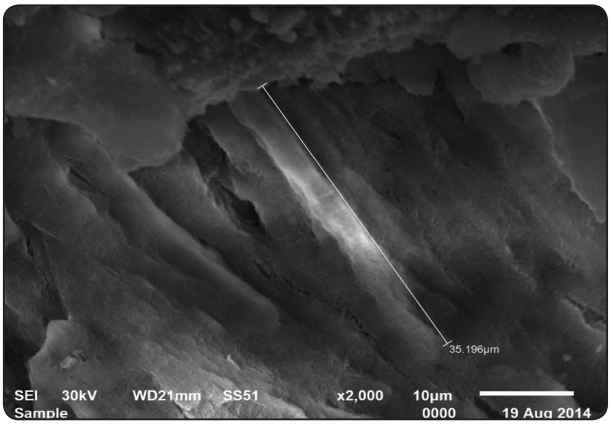

Fig. (d): Micrograph of a sample in subgroup IV showing the greatest sealant penetration into enamel pores.

TABLE (1) Effect of fluoride application on microleakage of pit and fissure sealant through different etching times.

Groups		Scores				Chi square test	p-value
		0	1	2	3		
Test	Subgroup I	6 (60%) Abc	2 (20%) ad€	0 bde	2 (20%) ce€#	57.29	0.00*
	Subgroup II	7 (70%) Abc	1 (10%) ad© #	0 bde	2 (20%) ce©&	81.66	0.00*
	Subgroup III	7 (70%) Abc	3 (30%) ade©&	0 Bd	0 ce€©•	98.41	0.00*
	Subgroup IV	8 (80%) Abc	2 (20%) Ade•	0 Bd	0 ce#&®	118.05	0.00*
Control	Control	9 (90%) Abc	0 ad€#&•	0 Be	1 (10%) cde•®	147.12	0.00*
p-value		0.406	0.00*	-	0.00*		

The same letters indicate significant differences between the scores within the same group
Same symbols indicate significant differences between the groups within the same score.
**** Statistical significant difference at p≤ 0.05***

Fig. (e) Microleakage score 0 Fig. (f) Microleakage score 1 Fig. (g) Microleakage sore 3

DISCUSSION

It was known that addition of fluoride to a sealant, or to the enamel prior to sealant application could have the benefit of additional caries prevention. Also it reduces the solubility of enamel without compromising sealant properties.[23]

Fluoride forms calcium fluoride, which affects the attachment of acid etching or sealant application. It was reported that an APF pretreatment led to decreased surface roughness in the acid-etched enamel in both the primary and permanent teeth. After the APF treatment, the more roughness of both the primary and permanent teeth increased with increasing time elapsed. Therefore, acid-etching should be performed two weeks after fluoride application to achieve the maximum enamel adhesion of a sealant. Prolonged acid-etching time (40 s) showed increased nanostructural changes in the enamel surfaces regardless of the time elapsed after the APF pre-treatment compared to the groups etched for 20 s. This suggests that after the APF treatment, even if two weeks have not elapsed, a prolonged acid-etching time can induce high surface roughness[14]. Therefore this study was performed to evaluate the effect of fluoride varnish application on acid etching before sealant application.

The results of the present study indicated that fluoride application prior to acid etching significantly affected the length of the formed enamel tags in the different groups with subgroup I being the least formed tags and nearly comparable tags in subgroups III and IV. Group IV showed the tallest formed enamel tags. These results indicated that the effect of fluoride on enamel etching decreased by increasing the etching time and it may resemble non fluoridated enamel after 60-120 seconds. Many previous studies reported that the effect of fluoride decreases with time although they reported that the enamel retained its normal etching pattern after different periods that ranged from 60 sec up to 4 minutes [14-17,24,25]. The variation in time elapsed till the etched enamel returns to the non-fluoridated pattern may be attributed to the difference in the etching agents and the concentration of the agent used. Additionally, the fluoride agents used in these studies may also contribute to the difference in variation in the etching time. Most of these studies used (APF) acidulated phosphate fluoride gel or sodium fluoride solution, while in this study we used Duraphat fluoride varnish. There are no in reach studies that used fluoride varnish before sealant application to compare the results.

The scanning examination revealed that sealant in all subgroups was in close contact with the enamel surface. These results suggested that the adhesion of the nano sealant used in this study was

not affected by the application of fluoride varnish before etching. This finding may explain a lot the reduced microleakage observed in this study. These results are in agreement with the results of Wang and Sheen[11], Kimura et al[13], and Cheong et al[14], who reported that the tensile strength of the sealant was not affected by the fluoride application before acid etching. However, it contradicts the results of Meng et al[12] who reported that the fluoride application before acid etching negatively affected the retention of the sealants.

One of the most important factors for the success of sealants is the marginal integrity of the material which indicates absence of microleakage. The results of this study showed that there is no significant difference between the control and test groups regarding score 0 (no microleakage. This finding is matching with the previous studies of Mirkarimi et al.2012[26]; Warran et al.2001[27]; and Garcia-Goday et al. 1991[28].

These results revealed that the use of fluoride prior to sealant application does not increase microleakage. Microleakage test was done using 2% buffered methylene blue dye. It is believed that when a material behaves positively (no or little leakage) in in vitro tests using dye stain, it would behave more better clinically because the penetration of the dye material is much easier than the bacteria and bacterial product[29].

On the other hand, this result can be attributed to the type of sealant used in this study. The nano sealant has better adaptation than conventional one due to the addition of nano particles which allow the production of flowable material with better flow ability and surface tension, so it can penetrate deep into etched enamel[30].

On the light of this study it can be concluded that application of fluoride did not affect the sealant application especially for the etching times 40 and 60 seconds.

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