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Original Article

Effects of sealant, viscosity, and bonding agents on microleakage of fissure sealants: An *in vitro* study

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of enamel or dentin bonding agent (DBA) and sealant viscosity on sealant microleakage. **Materials and Methods:** Sixty extracted human premolars were randomly divided into two equal groups (based on sealant viscosity) and each group was divided into three subgroups of 10 teeth. Group 1 (low viscosity sealant, Seal-Rite, Pulpdent, USA with 7.7% filler): Prophylaxis, enameloplasty, etching of occlusal surfaces with 38% of phosphoric acid gel, rinsing and drying, followed by (1) enamel bonding agent (EBA) (Margin Bond, Coltène/Whaledent AG) or (2) DBA (Excite, Ivoclar Vivadent AG, Liechtenstein) or (3) no bonding (NB) prior to sealant application. In Group 2, similar procedures were performed except for applying a high viscosity sealant (Seal-Rite, Pulpdent, The USA with 34.4% filler). Specimens were thermocycled and then immersed in a 0.5% basic fuchsine solution for 24 h next, buccolingual slices of samples were scored under a stereomicroscope. The Kruskal–Wallis and Mann–Whitney U-tests were used for data analysis. **Results:** There was no significant difference between DBA, EBA, and NB subgroups in the microleakage scores in both groups. Low viscosity sealant had a lower microleakage than the high viscosity sealant in both DBA ($P = 0.002$) and NB ($P = 0.041$) subgroups. **Conclusion:** The results indicated that the use of low viscosity sealant reduced the microleakage of pit and fissure sealants. However, the use of a bonding agent before sealant placement didn't affect the microleakage.

Key words: Dentin bonding agent, enamel bonding agent, microleakage, sealant viscosity

INTRODUCTION

During the past several decades, caries incidence in pediatric and adolescent groups has dramatically decreased, especially with respect to smooth surface lesions. However, despite this improvement, dental caries are particularly common on the surfaces with pits and fissures. To overcome this problem sealant – a material placed into caries-susceptible pits and fissures – is used to prevent the access of cariogenic bacteria to the source of nutrients. Thus, pit and fissure sealant could be a safe and effective way to prevent dental caries.[1] However, failure to use sealants correctly can cause leakage with partial or total loss leading to sealant failure at a rate of 5–10% years.[2]

So far, several studies have been done focusing on improving the success of fissure sealants.[3,4] An important factor to consider in the success of sealants is the prevention of microleakage, the ingress of bacteria and oral fluids into the space between the

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tooth and restorative material. Since microleakage can result in caries lesion development underneath the sealant, inhibiting marginal leakage is critical for sealant success. It has been shown that the application of the intermediate bonding layer between enamel and sealant after contamination of etched enamel with saliva improves result.[5] Other studies suggest that the application of the bonding agent before sealant improves bond strength and decreases microleakage,[6,7] although some studies have shown no such differences.[8-10]

There are two main groups of bonding agents: Enamel bonding agent (EBA) (hydrophobic agent) and dentin bonding agent (DBA) (hydrophilic agent). Given that the pit and fissures of occlusal surfaces consist mostly of enamel, it seems that it is better to use EBA than DBA, but there is no conclusive evidence to support it.

Some investigations have shown that sealant viscosity may adversely affect the success of fissure sealant.[11] However, there are some contraindicatory findings of the effect of sealant viscosity. Some studies have indicated that compared to high viscosity resin, low viscosity sealant material exhibits better marginal adaptation.[12-15] On the other hand, other studies have reported no difference between unfilled and filled sealants regarding microleakage scores.[16]

The aim of this study was to evaluate the effect of enamel bonding, dentin bonding, and sealant viscosity on microleakage of fissure sealant.

MATERIALS AND METHODS

This study was carried out at the Pediatric Department of the Dental School with the permission of the Research and Ethics Committee, School of Dentistry, University of Medical Science (Mashhad, Khorasan, Iran).

In this *in vitro* study 60 human maxillary premolars extracted for orthodontic reasons were used. All teeth were cleaned and stored in 0.2% of thymol solution at room temperature. After cleansing with a rubber cup and pumice powder, occlusal fissures were slightly opened using a fissurotomy bur (Fissurotomy® Original - FGSS SS White, Lakewood, NJ USA) on a high-speed handpiece. The teeth were randomly assigned to two equal groups based on the type of sealant viscosity, and each group was divided into three subgroups of 10 teeth.

Group 1 (subgroup 1): The occlusal pit and fissures were etched (Etch Rite, Pulp Dent, USA) for 20 s, and then rinsed for 15 s and thoroughly air dried with oil-free compressed air to obtain a white, dull and chalky-like appearance. Next, a low filler sealant of 7.7% w/w (Seal-Rite, Pulp Dent, USA) was applied, and light-cured for 40 s.

Group 1 (subgroup 2): The teeth were conditioned similarly to the previous subgroup, but prior to low filler sealant placement, EBA (Margin Bond, Coltène/Whaledent AG) was applied with a hand-held brush, air-thinned after 10 s and light cured for 20 s.

Group 1 (subgroup 3): The procedure was similar to Group 1 (subgroup 2), except instead of EBA, DBA (Excite, Ivoclar Vivadent AG, Liechtenstein) was applied before sealant placement according to the manufacturer's instructions.

Group 2 and its subgroups went through similar processes, except a high filler sealant of 34.4% w/w (Seal-Rite, Pulp Dent, USA) was used.

Polymerization was performed using a halogen light curing unit (Coltolux 75, Coltene/Whaledent) with an output of 500 mW/cm^2. The light intensity was checked periodically with the digital radiometer (Coltolux®, Coltène/Whaledent Inc.,). All specimens were stored in distilled water at 37°C for a week, thermocycled for 500 cycles between 5°C (±2) and 55°C (±2) with a 10 s dwell time at each temperature. All teeth surfaces, except for the 1 mm outside the margins of the sealant, were double coated with nail varnish and were immersed in a 0.5% basic fushin dye solution for 24 h. Following immersion, all teeth were washed under running tap water for 30 s to remove excess dye solution, and then embedded in epoxy resin and sectioned in the buccolingual direction at mesial and distal pits with a water-cooled diamond disk (Isomat 2000, Buehler, Lake Bluff, IL, USA) to create three sections and four surfaces per tooth, each approximately 1.5 mm in thickness.

For microleakage evaluation, one trained and blinded examiner scored the dye penetration depth in each section using a stereomicroscope (Wild, Leitz Ltd., Herbage, Switzerland) at ×40. Thus, the degree of microleakage judgment was kept blind.

The scoring system used in the present study was as follows:

- Score 0: No dye penetration
- Score 1: Dye penetration limited to the outer half of the sealant

- Score 2: Dye penetration to the inner half of the sealant
- Score 3: Dye penetration extends into the bottom of the fissure.

This evaluation scale is the most widely used in previous studies on sealant microleakage.[17] Where scores were different at four surfaces per tooth, the worse score (higher degree of leakage) was referred to as the leakage score.

To determine statistically significant differences in leakage among the subgroups in the two groups (low or high viscosity) separately, nonparametric data were analyzed using the Kruskal–Wallis test. The Mann–Whitney U-test was performed to compare the leakage between the two viscosities at each subgroup separately. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago. IL., USA) at the significance level of $P < 0.05$.

RESULTS

The present study aimed to assess comparatively the microleakage of teeth sealed with high or low filled sealants (Seal-Rite) with and without the application of bonding agents (Exite or Margin Bond).

Hence, 60 teeth (240 surfaces) were evaluated in this study for microleakage. The frequency distribution of microleakage between various groups is shown in Table 1. At the low viscosity group, EBA subgroup had the maximum microleakage, followed by no bonding (NB) subgroup; and, minimum microleakage was found in DBA subgroup. At the high viscosity group, microleakage of NB subgroup was higher than the two other subgroups, but Kruskal–Wallis test revealed no statistically significant difference between the three subgroups of low viscosity ($P = 0.114$) or high viscosity sealant groups ($P = 0.648$) [Table 1].

Table 1: Distribution of microleakage scores of low and high viscosity sealant groups

Group	Sub group*	Microleakage score number (%)				*P* value
		0	1	2	3	
Low viscosity sealant	NB	8(80)	0(0)	0(0)	2(20)	0.114
	EBA	6(60)	3(30)	0(0)	1(10)	
	DBA	10(100)	0(0)	0(0)	0(0)	
High viscosity sealant	NB	2(20)	3(30)	2(20)	3(30)	0.648
	EBA	4(40)	1(10)	1(10)	4(40)	
	DBA	3(30)	4(40)	2(20)	1(10)	

*NB: No bonding; EBA: Enamel bonding agent; DBA: Dentin bonding agent

The Mann–Whitney U-test showed a significant difference between the two viscosities in the NB subgroup ($P = 0.041$). Low viscosity sealant showed better marginal integrity than the other group [Figure 1].

In the EBA subgroup, there was no significant difference between the two viscosities. (Mann–Whitney U-test, $P = 0.171$).

Comparison of the microleakage scores in the DBA subgroup showed a significant difference between the two viscosities (Mann–Whitney U-test, $P = 0.002$). Microleakage at the low viscosity sealant group was significantly lesser than the high viscosity sealant group [Figure 2].

DISCUSSION

Use of fissure sealant after acid etching has been advocated as a reliable method for the prevention of fissure caries. There are several factors that may affect sealant success and among those are the effects of sealant viscosity and bonding agent application on marginal sealing. This study was based on a comparative assessment of microleakage of teeth sealed with high or low filled sealants with and without the application of bonding agents.

Effect of bonding agent application

The results indicate that in both high and low viscosity sealant groups, application of bonding and its type had no effect on microleakage. In the high viscosity sealant group, it was expected that using bonding (EBA or DBA) would result in better penetration and lower microleakage, but that was not the case. It seems that in spite of high viscosity, it still has enough flowability to penetrate into the microspaces and form resin tags. Kwon's study also showed that applying high viscosity sealant (60% filled) without any bonding could result in resin tag formation and low microleakage.[18] In an *in vivo* study, Boksman *et al*.[19] compared the retention rate of a filled and an unfilled sealant with and without using DBAs. After 2 years, they reported that using bonding agents did not increase the retention of either type of sealants.

However, in the low viscosity sealant group, it seems that the high flowability of the sealant is the main factor in reducing leakage and adding an intermediate layer of bonding agent has no additional effect on improving the marginal seal. It has been shown that the degree of penetration of a sealing agent into the interfacial micro-gap directly corresponds to the material's viscosity and potential to adequately wet

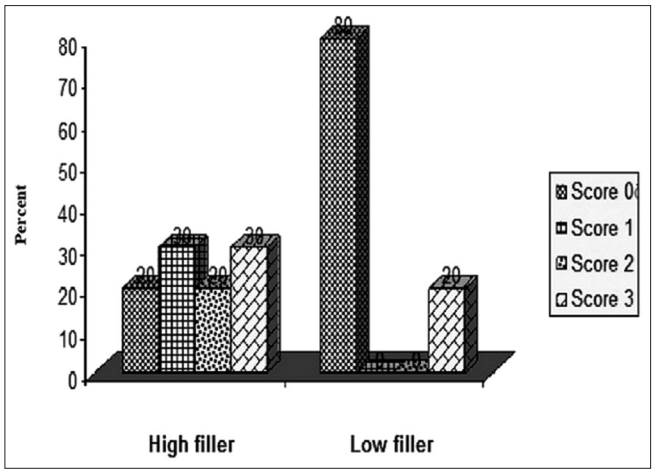

Figure 1: Distribution of microleakage scores in no bonding subgroup according to sealant viscosity

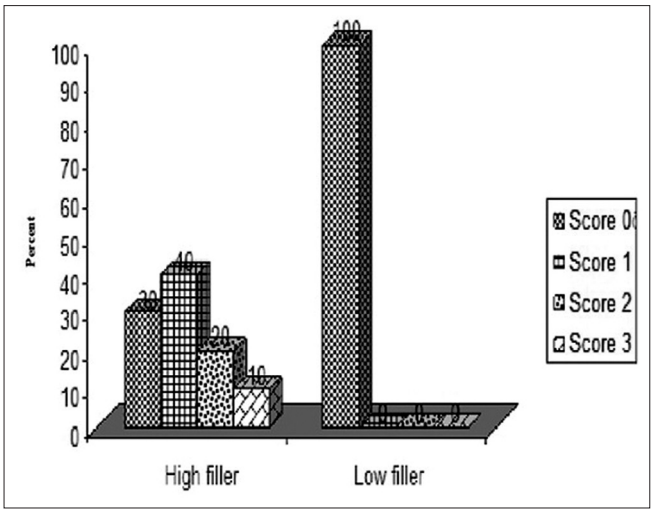

Figure 2: Distribution of microleakage scores in dentin bonding agent subgroup according to sealant viscosity

restoration or tooth surfaces.[20] Percinoto *et al*. also mentioned that low viscose sealant had a greater potential to penetrate into the fissures and the micro porosities produced in the enamel by etching.[21]

A review article by Das and Suma[10] showed that using bonding agent as an intermediary layer between enamel and sealant did not affect sealant success, except in cases in which adequate isolation and contamination control is impossible. As this was an *in vitro* study and there was no saliva contamination, it could be expected that the application of any bonding agent has no statistically significant effect on sealant microleakage. Also, other clinical studies[22,23] revealed no significant differences with or without using bonding agents prior to sealant application. It seems that the success of a sealant is related to whether the sealant is applied under optimal conditions. The two latter studies have been performed in a dry and isolated situation, so the results are predictable.

Effect of sealant viscosity

The results showed that in the NB subgroup the low viscosity sealant had significantly lower microleakage. This result is in agreement with the results of other studies. Irinoda *et al*.[13] and Prabhakar *et al*.[24] concluded that low viscosity sealant can penetrate fully into the etched surface and form a resin-infiltrated layer in enamel beyond the etched depth, whereas the high viscosity sealant cannot penetrate enough to provide good marginal seals. Kakaboura *et al*. reported that unfilled sealant penetrated deeper in narrow fissures than low viscosity resin composite and compomer.[25] On the other hand, Barnes *et al*.[26] and Droz *et al*.,[27] concluded that although the penetration of different commercial sealant products is different, viscosity and flow properties of a sealant have no effect on sealing capacity. This is parallel to the result of Breakspere and Wilton, which have shown that effective attachment of the sealant to enamel required to be brushed into the dry etched enamel and sealant viscosity seems unimportant.[28] Other investigators such as Park *et al*. reported that unfilled and filled sealants exhibited no differences regarding the microleakage score.[16]

The low viscosity sealant also showed better results in the DBA subgroup, so that the combination of DBA and low viscosity sealant resulted in the samples not exhibiting any dye penetration. Better results in this subgroup than the NB subgroup could be partly explained based on the impact of the increased surface moisture-chasing effect of the hydrophilic primers (HEMA), increased flow as a result of the less viscous primer, and increased flexibility of polymerized bonding agent or sealant complex due to application of DBA.[29]

In the EBA subgroup, we expected the same result as the DBA subgroup, but there was no significant difference between the two sealants. The findings may be due to the fact that the EBA (Margin Bond®) used in this study was an unfilled hydrophobic adhesive with lack of a primer to increase its wetting ability, and its fluidity was less than the used DBA. Hence, it seems that it leads to better results in the high viscosity sealant (the two samples with a score of 0 changed to 4 samples without microleakage as shown in Table 1). But, there were no good results in the low viscosity sealant group (the eight samples without

microleakage changed to six samples with a score of 0 as shown in Table 1). Therefore, the dissimilarities between the two viscosities have been reduced so that there was no significant difference between the two sealants in the EBA subgroup.

Lack of access to a cutting machine with more delicate blades that would allow us to provide more slices per tooth for a more detailed microleakage assessment was one limitation in this study. Also, like other *in vitro* studies, the results need to be supported with clinical studies.

This study showed that low viscosity sealant was superior because it showed less microleakage when compared to the high viscosity sealant. However, the use of a bonding agent prior to the application of a pit and fissure sealant does not decrease the microleakage rate.

CONCLUSIONS

Based on the conditions in our study, the following conclusions were made:

- The Seal-Rite sealant with low filler particles acts better than Seal-Rite with high filler particles as a pit and fissure sealant material regarding microleakage
- The use of a bonding agent (DBA or EBA) prior to the application of a pit and fissure sealant does not decrease the microleakage rate.

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Conflicts of interest

There are no conflicts of interest.

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