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Research Article

Effect of Different Protocols on The Microleakage of A Fissure Sealant Applied During Saliva Contamination

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Abstract

Statement of the problem: Saliva contamination during sealant application has negative consequences that affects long-term success including retention and caries progression.

Objective: The effect of different protocols to minimize the effect of saliva contamination on the microleakage of a resin based fissure sealant material (3M Clinpro™ Sealant) was investigated.

Materials & Methods: Extracted human third molars (n=160) were used: Group A. Etch-and-rinse adhesive (Prime & Bond One Select); Group B. Self-etching adhesive (Clearfil SE Bond). These comprised eight paired subgroups where enameloplasty was done (or not), saliva contamination occurred before (or after) the polymerization of the bonding agent and the entire procedure was repeated (or not) following saliva contamination before the sealant application. Following thermocycling, the samples were immersed in basic fuchsin, sectioned, and dye penetration was quantitatively assessed with ImageJ. The data were statistically analyzed (α=0.05).

Results: Significantly less microleakage was observed in Group A (*P*=0.000). Intergroup differences with respect to the effect of adhesive type, enameloplasty, saliva contamination occurred **after** the polymerization of the bonding agent and repeating the entire procedure following saliva contamination were significant ($p<0.05$, each). Enameloplasty reduced microleakage in subgroups of A and B (*P*=0.002 and *P*=0.014, respectively). Saliva contamination after the polymerization of the bonding agent resulted in less microleakage in subgroups of A and B (*P*=0.01 and *P*=0.002, respectively). Less microleakage was observed in subgroups of A and B where the entire procedure was repeated following saliva contamination (*P*=0.000, both).

Conclusions: The use of etch-and-rinse adhesive, enameloplasty, saliva contamination occurring after the polymerization of the bonding agent and repeating the entire procedure reduced microleakage of the fissure sealant applied during saliva contamination.

Keywords: Etch-and-rinse **Adhesive**, Microleakage, Pit and Fissure Sealant, Saliva Contamination, Self Etching Adhesive

Introduction

Resin-based sealants are the most effective means for preventing caries on occlusal surfaces of primary and permanent molars in children and adolescents.[1] Although pits and fissures of occlusal surfaces comprise 12.5% of total tooth surfaces, they account for 88% of coronal caries prevalence in schoolchildren.[2] Resin-based sealants reduce caries by 11% to 51% compared to no sealant after 24 months.[1] However, the effectiveness is directly related to the retention which is dependent upon a meticulous method of application.[3] Due to their hydrophobic characteristics, these materials should be applied with sufficient moisture control. However, this is not always attainable, particularly in children with low compliance and when rubber-dam is not used.

Acid etching creates microporosities that serve for the marginal integrity and retention of the sealant material by increasing its bond strength to the enamel.[4] However, saliva contamination of the conditioned enamel leads to inadequate adhesion (gap formation),[3,5] loss of fissure sealant, or formation of secondary caries due to microleakage.[6,7] The use of a hydrophilic adhesive resin as an intermediate layer under the sealant material has been proposed to overcome these consequences.[6,8] Improved microleakage resistance was reported for etch-and-rinse adhesives, whose utilization also led to higher micromechanical bond strengths.[9-11] Fissure sealants placed with etch-and-rinse adhesives also showed better retention than those placed with self-etching adhesives.[8,12,13]

Without the need for separate etching, rinsing, and drying steps, self-etching adhesives offer ease of use.[14] This may also help reduce the risk of contamination, especially in pediatric patients difficult to cooperate. However, these type of adhesives were claimed to provide insufficient bond strengths to unground enamel.[15] Only with prior enamel etching, they were able to improve the retention of a resin sealant.[13]

The occlusal fissures are considered resistant to etching due to the ring of aprismatic enamel surrounding the entrance and walls of fissures.[16,17] The enameloplasty carried out before surface conditioning decreased the microleakage of applied fissure sealants.[18] Hence, the procedure may also help improve the bonding of self-etching adhesives to aprismatic enamel as they are not able to dissolve this layer of enamel.[19]

The effect of saliva contamination occurring at different stages of adhesive bonding has been investigated.[20,21] Saliva contamination before and after the polymerization of the adhesive did not affect microleakage of Class V composite restorations bonded with either a two-step etch-and-rinse or a one-step self-etching adhesive.[21] However, this area has not been explored for fissure sealants applied with different adhesive systems.

The present study aimed to investigate the effect of different protocols on the microleakage of a resin-based fissure sealant applied under saliva contamination. The tested null hypotheses were as follows:

1.The type of adhesive system has no effect on the microleakage of the fissure sealant.

2.Enameloplasty has no effect on the microleakage of the fissure sealant.

3.Saliva contamination occurring before or after the polymerization of the adhesive does not affect the microleakage of the sealant.

4.Repeating the entire procedure following saliva contamination does not affect the microleakage of the sealant.

Materials & Methods

The study protocol was approved by the Non-interventional Clinical Researches Ethics Board of Hacettepe University in Ankara, Turkey (No: 2019/05-09). Freshly extracted 160 human third molars were collected following the patients' consents. After surface debridement, pits and fissures were cleaned with a low-speed water-cooled rotating brush and non-fluoride prophylaxis paste. The teeth were examined under a stereomicroscope (Olympus SZ61, Tokyo, Japan) at 20X to exclude the ones with caries, surface cracks, or developmental defects. Until their use, the teeth were stored in dilute sodium azide solution at 4 °C to prevent bacterial growth.

A 2X2X2X2 study design was employed to test the effect of four parameters on the microleakage of a resin-based fissure sealant material, 3M™ Clinpro™ Sealant (3M, St. Paul, USA) applied during saliva contamination. These were

1. The use of an etch-and-rinse (Prime&Bond® One Select; Dentsply Sirona Konstanz, Germany) or a self-etching (Clearfil™ SE Bond; Kuraray, Okayama, Japan) adhesive system
2. Sealant application with or without prior enameloplasty
3. Saliva contamination occurring before or after the polymerization of the bonding agent
4. Repeating or not repeating the entire procedure following saliva contamination

The manufacturers' instructions were followed during application of the study materials. (Table 1).

Table 1. Composition of the materials used in the study.

Product	Manufacturer	Composition
3M Clinpro™ Sealant	3M St. Paul, Minnesota, USA	TEGDMA, Bis-GMA, Silane treated silica, Tetrabutylammonium tetrafluoroborate, Diphenyliodonium hexafluorophosphate, Triphenylantimony, EDMAB, Titanium Dioxide, Hydroquinone
Prime&Bond® One Select	Dentsply Sirona Konstanz, Germany	PENTA, TEGDMA, bis-GMA, Di and trimethacrylate resins, functonal amorphous silicate, cetylamine hydrofluoride, acetone, photoinitiators
Clearfil™ SE Bond	Kuraray, Okayama, Japan	Primer: MDP, HEMA, Hydrophilic dimethacrylate, water Adhesive: MDP, bis-GMA, HEMA, dimetachrylate, silanated colloidal silica
I-Gel	I-Dental, Siauliai, Lithuania	37% orthophosphoric acid

Abbreviations: Bis-GMA: Bisphenol A diglycidyl ether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; UDMA: Urethane dimethacrylate; PENTA: dipentaerythritol penta acrylate monophosphate; EDMAB: Ethyl 4-dimethyl aminobenzoate; DMA: Diurethane dimethacrylate.

In eight subgroups where enameloplasty was performed, a diamond bur (Diatech Dental Instruments) for high-speed instrument was used. It had a very fine tapering tip that enabled to avoid unnecessary enlargement of the fissures.17 The procedure was carried out for 1-2 minutes with a sweeping motion on the occlusal surfaces. Care was taken not to penetrate the dentin, and the bur was changed in each subgroup.

For contamination of the prepared occlusal surfaces, unstimulated human saliva was used. It was freshly collected from the same single individual (one of the researchers) at least 1 hour after the consumption of any food or drink before the procedures in each subgroup. One drop (0.025 ml) was left undisturbed on the surface for 20 seconds.22 The contamination and decontamination procedures simulated the clinical situation during the process of restoration. A detailed description of the procedures in each group is presented in Table 2.

Table 2. Detailed application protocol of the study materials

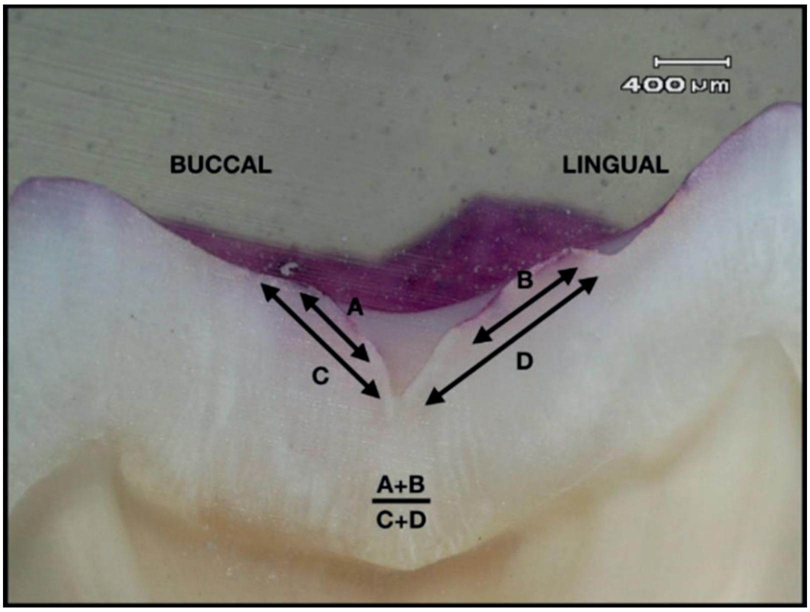
A	**Enameloplasty** • Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	A 1	• No re-conditioning • Apply sealant and polimerize
				A 2	• Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s • Apply sealant and polimerize
	Enameloplasty • Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	A 3	• No re-conditioning • Apply sealant and polimerize
				A 4	• Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s • Apply sealant and polimerize
	No Enameloplasty • Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	A 5	• No re-conditioning • Apply sealant and polimerize
				A 6	• Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s • Apply sealant and polimerize
	No Enameloplasty • Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	A7	• No re-conditioning • Apply sealant and polimerize
				A8	• Apply 37% phosphoric acid for 30 s. • Rinse off with water spray for 30 s • Air-dry for 10 s • Apply Prime&Bond One Select wait for 20 s, air-blow for 5 s • Light cure for 10 s • Apply sealant and polimerize

Table 2. (continued)

B	**Enameloplasty** • Apply the primer of Clearfil SE Bond, wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	B1	• No re-conditioning • Apply sealant and polimerize
				B2	• Apply the primer of Clearfil SE Bond wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s • Apply sealant and polimerize
	Enameloplasty • Apply the primer of Clearfil SE Bond, wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond air-blow gently	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	B3	• No re-conditioning • Apply sealant and polimerize
				B4	• Apply the primer of Clearfil SE Bond wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s • Apply sealant and polimerize
	No Enameloplasty • Apply the primer of Clearfil SE Bond, wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	B5	• No re-conditioning • Apply sealant and polimerize
				B6	• Apply the primer of Clearfil SE Bond wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s • Apply sealant and polimerize
	No Enameloplasty • Apply the primer of Clearfil SE Bond, wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond air-blow gently	Natural Saliva	• Rinse off with water spray for 30 s • Air-dry for 10 s	B7	• No re-conditioning • Apply sealant and polimerize
				B8	• Apply the primer of Clearfil SE Bond wait for 20 s, air-blow gently • Apply the bond of Clearfil SE Bond, air-blow gently • Light cure for 10 s • Apply sealant and polimerize

After polymerization of the sealant, the specimens were stored in distilled water at 37 °C for 7 days. They were then subjected to thermocycling for 1000 cycles, in 5 ± 2°C to 55 ± 2°C with a dwell time of 15 s and a transfer time of 10 s. Their apices were sealed with sticky wax, Occlusal surfaces were painted with two coats of nail varnish in order to leave 1 mm space around sealant margins. They were then immersed in 0.5% basic fuchsin solution (Wako Pure Chemical Industry; Osaka, Japan) at 37°C for 24 hours. After that, the specimens were thoroughly rinsed with distilled water; nail varnish and sticky wax were removed with a sharp instrument. The specimens were embedded in chemically activated acrylic resin (Integra, BG Dental, Turkey) and four sections of 0.5 mm thickness were obtained from each using a slow-speed, water-cooled diamond saw (Micracut 201, Metkon, Bursa, Turkey).23 A digital photograph of each section was taken at 20X under a stereomicroscope (Olympus SZ61, Tokyo, Japan). They were transferred to a Macintosh PowerPC workstation. One calibrated operator, blinded to treatment groups, using an open-source image analysis software (ImageJ for MacOSX; V.1.34, National Institutes of Health; Bethesda, MD, USA) measured the extent of buccal and lingual dye penetration along the enamel/fissure sealant interface (in mm).24 The microleakage value for each section was calculated by dividing the total of buccal and lingual dye penetration values by the total of the lengths of buccal and lingual enamel-fissure sealant interfaces (Figure 1).

Figure 1. Scoring system for the evaluation of microleakage (modified from Duangthip and Lussi24). A + B (mm) = length of dye penetration along the buccal and lingual walls. C + D (mm) = length of fissure sealant-tooth interface. A + B / C + D = mean microleakage value for the section

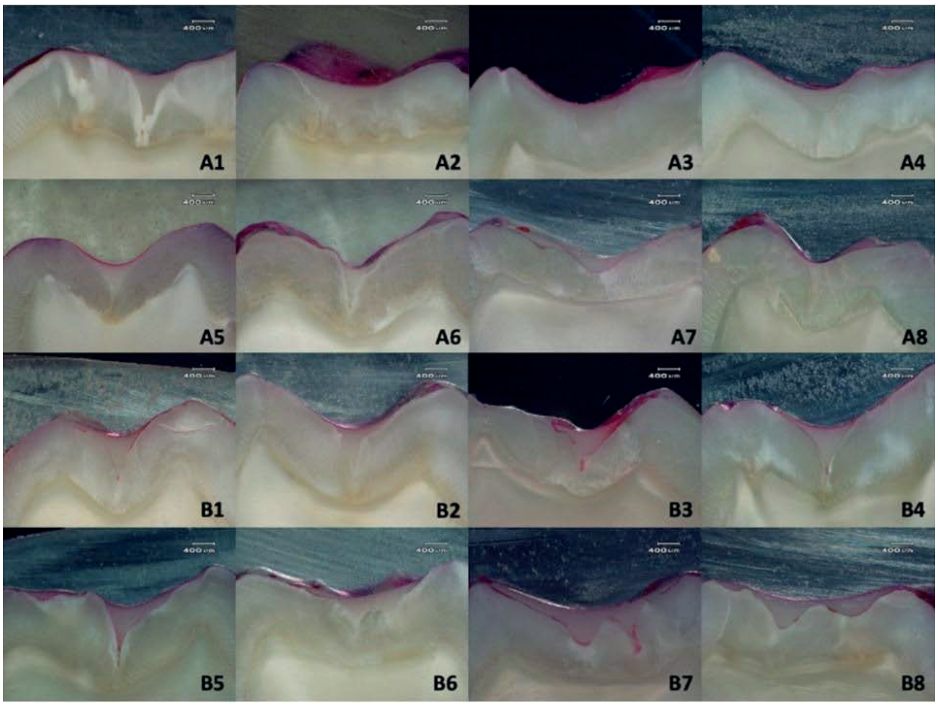


For all statistical analyses, Statistical Package for Social Science 11.5 software for Windows (SPSS®, SPSS Inc., Chicago IL, USA) was used. The level of significance was set as $\alpha = 0.05$. Descriptive statistics such as mean and standard deviation for normally distributed numerical variables and median and interquartile range of distribution for non-normally distributed numerical variables were given. The data were analyzed using two-way ANOVA for the application of the protocols and another two-way ANOVA for repeating the entire procedure. Mann-Whitney U test was used for intergroup comparisons in Groups A and B, along with Bonferroni post-hoc test. The assumption of normality for the numerical variable was examined using the Shapiro-Wilk test statistics.

Results

A total of 640 sections were prepared in 160 teeth. Microleakage measurements were completed on 617 sections since 23 sections were found unsuitable for evaluation and were excluded. A selection of photographed sections is presented in Figure 2.

Figure 2. Stereomicroscopic views of microleakage observed in subgroups



The microleakage values obtained in the study are presented in Table 3.

Table 3. Mean and standard deviation of fissure sealant microleakage for two types of adhesives, two types of surface preparation, two types of contaminaton and two types of application following contamination

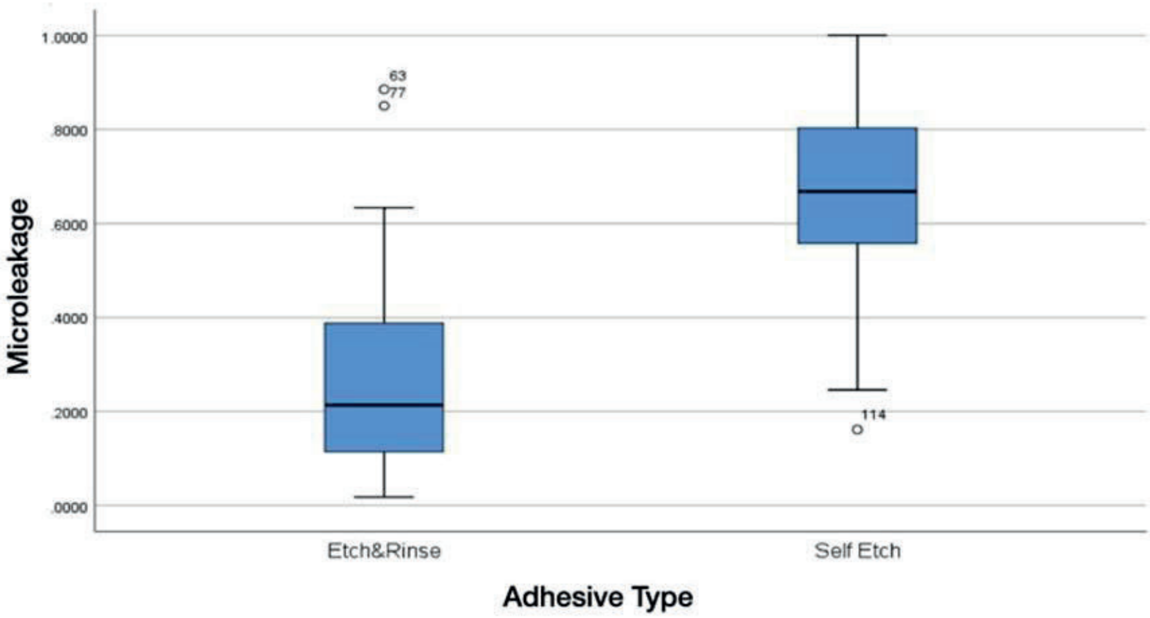
Table 3. Mean and standard deviation of fissure sealant microleakage for two types of adhesives, two types of surface preparation, two types of contaminaton and two types of application following contamination*

			The Procedures Were Not Repeated Following Saliva Contamination			The Procedures Were Repeated Following Saliva Contamination		
Adhesive	Ename-loplasty	Saliva Contamination	N	Subg-roup	Microlea-kage	N	Subg-roup	Microlea-kage
Group A Prime&Bond® One Select	Yes	After Polymerization	10	A1	0.22±0.10	10	A2	0.08±0.06
		Before Polymerization	10	A3	0.33±0.15	10	A4	0.11±0.05
	No	After Polymerization	10	A5	0.35±0.17	10	A6	0.15(0.19)
		Before Polymerization	10	A7	0.56±0.13	10	A8	0.21(0.13)
Group B Clearfil™ SE Bond	Yes	After Polymerization	10	B1	0.58(0.12)	10	B2	0.50±0.08
		Before Polymerization	10	B3	0.78±0.17	10	B4	0.58±0.18
	No	After Polymerization	10	B5	0.69±0.15	10	B6	0.65(0.13)
		Before Polymerization	10	B7	1.00(0.13)	10	B8	0.64±0.12

* Values are presented as "mean±standard deviation" for groups with variables normally distributed, and as "median (interquartile range)" for groups with variables not normally distributed.

The intergroup comparisons showed significantly less microleakage in Group A (etch-and-rinse adhesive) than in Group B (self-etching adhesive) (P=0.000) (Figure 3).

Figure 3. Box-Plot graph of microleakage amount according to the adhesive types used



In enameloplasty subgroups (A1, A2, A3, A4, B1, B2, B3, B4), microleakage was significantly less than the subgroups where this procedure was not employed (A5, A6, A7, A8, B5, B6, B7, B8) (P=0.000 for adhesive type, P=0.000 for enameloplasty and P=0.379 for their interaction). In subgroups where saliva contamination occurred after the polymerization of the bonding agent (A3, A4, A7, A8, B3, B4, B7, B8), less microleakage was observed than in subgroups where contamination was before the polymerization (A1, A2, A5, A6, B1, B2, B5, B6) ($p<0.01$ for adhesive type, $p<0.01$ for saliva contamination occurring after the polymerization and P=0.873 for their interaction). Microleakage in subgroups where the procedures were repeated after saliva contamination was significantly lower (A2, A4, A6, A8, B2, B4, B6, B8) than subgroups where the procedures were not repeated after the contamination (A1, A3, A5, A7, B1, B3, B5, B7) ($p<0.01$ for adhesive type, $p<0.01$ for repeating the entire procedure after saliva contamination and P=0.475 for their interaction).

With regard to intragroup comparisons in Group A and B, enameloplasty resulted in less microleakage in subgroups A1, A2, A3, A4 (P=0.002) and B1, B2, B3, B4 (P=0.014) than subgroups with no enameloplasty (A5, A6, A7, A8 and B5, B6, B7, B8, respectively). Microleakage was less in subgroups where saliva contamination occurred after the polymerization of the adhesive (A3, A4, A7, A8 and B3, B4, B7, B8) than in subgroups where saliva contamination was prior to the polymerization of the adhesive (A1, A2, A5, A6 and B1, B2, B5, B6) (P=0.01 and P=0.002, respectively). The reapplication of entire procedure after saliva contamination improved the sealant's resistance to microleakage in subgroups A2, A4, A6, A8 and B2, B4, B6, B8 compared to the subgroups A1, A3, A5, A7 and B1, B3, B5, B7 where no such procedures were carried out (P=0.000 and P=0.000, respectively).

Discussion

Any restorative procedure requires a surface free of contaminants to achieve optimum adhesion.5,25 Surface conditioning (e.g. acid etching) prior to sealant application is an essential step.4 Saliva contamination is one of the most important factors that can disturb the sealing process and compromise the longevity of fissure sealants.26 It has been shown that contamination with saliva, for as short as one second, resulted in the formation of a surface coating that could not be removed effectively by rinsing.27

Microleakage tests are one of the methods to evaluate the sealing performance of dental materials.[23] Among other methods, measurement of dye penetration on sections of restored teeth is the most commonly used technique. In the present study, four sections were made through each sealant to increase the reliability of measurements.[23] This technique was combined with digital image analysis to obtain quantitative results instead of a conventional subjective scoring.[11] The relative merit of this objective approach, compared to a subjective scoring system, was to discard the need for scoring by separate evaluators, as well as statistical procedures involving interexaminer reliability.[24]

Etch-and-rinse adhesives have been reported to reduce the microleakage of fissure sealants that were applied following saliva contamination.[9,7,28] In this regard, the present study findings also favored the use of etch-and-rinse adhesive over the self-etching adhesive tested. Prime&Bond® One Select, is a universal adhesive that can be used in etch-and-rinse, selective etch, and self-etching modes.[29] Its etch-and-rinse mode was used in the present study. The solvent of the adhesive system is acetone. It is a "water chaser" whose boiling point increases and that of water decreases when it comes in contact with the moistened surface. Acetone and water then evaporate, leaving the resin behind.[30] Prime&Bond® One Select has also nano-fillers, a cross-linked molecule, T-resin, and D-resin, a small molecule of fluid. These resins and nanofillers have been reported to increase the adhesion to acid-etched dentine.[29]

Clearfil SE™ Bond contains a 2-hydroxyethyl-methacrylate (HEMA) monomer and a functional phosphate monomer, MDP (10-Methacryloyloxydecyl dihydrogen phosphate). The latter favors the diffusion process and improves adhesion to either dry or moist enamel.31 HEMA offers strength to cross-linking formed from the monomeric matrix. HEMA-containing adhesives are more vulnerable to moisture in saliva, as the HEMA in uncured adhesive tends to absorb water and end up diluting the monomers to the extent that polymerization is inhibited.32 The findings of the present study indicated that the type of adhesive affected the microleakage of the sealant applied under saliva contamination. Hence, the first null hypothesis was rejected.

Both intra, and intergroup comparisons indicated that enameloplasty reduced microleakage of the sealant in the present study, The second null hypothesis was rejected. The etch-resistant characteristic of aprismatic enamel in the occlusal fissures is a known phenomenon.[16] In addition to this, the etchant may not penetrate further than the fissure entrances even if the fissures are free of deposits as shown by Garcia-Godoy and Gwinnett[33] The limited depth of decalcification due to the questionable function of the etchant when it comes into contact with the core of the enamel prism results in a thin layer of resin and thin, lamina-like resin extensions.[15,34] Studies have reported that the mechanical preparation of pit and fissures created rough surfaces that could easily be wetted by the etching agents, helped remove plaque and debris;[35] thereby improved sealant penetration and retention.[36] In light of these findings, prior enameloplasty and the use of an etch-and-rinse adhesive could be suggested for situations that patient compliance poses a risk for adequate isolation during sealant applications.

The methacrylated phosphoric acid esters, also present in the tested self-etching adhesive, form shallow etching patterns on unground enamel.[14] An in vitro study comparing phosphoric acid and self-etching primers on intact enamel has shown that self-etching primers did not demineralize the enamel sufficiently and resulted in shorter resin tags as well as lower bond strength.[19] Additionally, dissolved calcium phosphates cannot be removed with self-etching adhesive systems since they are not rinsed. This has been reported to result in lower resistance to thermomechanical stress, and compromise marginal integrity of the fissure sealant.[15,19] Hence, another important aspect of the above-mentioned finding was that, even with enameloplasty, the self-etching adhesive was not equal to or better than the etch-and-rinse adhesive in terms of reducing microleakage.

Saliva contamination occurring at various stages of restoration had different influences on the bond strength of contemporary dental adhesives. Several studies reported significant adverse effects on the performance of the adhesives.[21,25] Nair *et al.,*[25] stated that when the tooth surface is contaminated after the application of adhesive, but before the polymerization, the degree of conversion may be affected. The hydrophilic molecules may retain water within the adhesive layer and disperse in water. Hence, they become unable to participate in chain growth during polymerization. This results in an alteration of the bond strength.[25] On the other hand, if contamination occurs after the polymerization of the adhesive, absorption of glycoproteins to the polymerized and air-inhibited adhesive surface may cause a reduction in bond strength. These glycoproteins prevent complete infiltration of the subsequent resin layer and co-polymerization.[37]

Dentine shear bond strengths (SBS) of a self-etching and universal adhesive were studied by Nair *et al.,*[22] who implemented saliva contamination after surface preparation, after primer application (for the self-etching adhesive), and after polymerization of the adhesives. They reported a detrimental effect of saliva contamination if it occurs after the application of the self-etching primer. Hitmi *et al.,*[20] also evaluated the changes in SBS of etch-and-rinse and self-etching adhesives on dentin surfaces prepared for composite resin bonding. They found different effects of the contamination occurring at different stages (i.e., before adhesive application, after adhesive application, and after polymerization of the adhesive). For etch-and-rinse adhesives tested, saliva contamination prior to the adhesive application had no adverse effect on the bonding efficacy. However, bond strength significantly decreased in all groups when saliva contamination occurred after the adhesive application. The self-etching primer (Clearfil Liner Bond 2) was not tolerant to saliva contamination if it occurred before the polymerization of the adhesive. The authors related these findings to the oxygen and water contained in saliva, which prevented the polymerization of the bonding agents.

In the present study, significantly reduced sealant microleakage was observed when saliva contamination occurred after the polymerization of the bonding agent. Methodological dissimilarities (enamel vs dentin surfaces; bond strength vs microleakage testing) and the lack of studies investigating this specific issue on enamel impeded direct comparison of the findings obtained. Hence, there is a need for future studies which should include this relatively new adhesive, Prime&Bond® One Select.

As a possible measure to reduce the sealant's microleakage, the present study examined the effect of reapplication of the entire procedure following saliva contamination. By using this protocol, the sealant's resistance to microleakage significantly improved in both groups. Hence, the fourth hypothesis was rejected. A similar result was reported by Şimşek *et al.,*[38] who used artificial saliva and found that both the etch-and-rinse adhesive and acid-etching only were successful when they were reapplied after saliva contamination. However, reapplication of the self-etching adhesive did not affect the microleakage of the fissure sealants tested. It should be noted that no prior enameloplasty was employed in that study. This might help explain the differences observed in these two studies.

A recent literature review by Nair et al[25] reported that 65% of the evaluated studies for decontamination achieved improved bonding when the contaminated surfaces were subjected to some kind of decontamination procedure. The authors used either "rinse and air-dry" or "rinse, air-dry and reapply the adhesive" as decontamination protocols.[22] They showed that the SBS was regained and maintained over time with the latter. In another study, additional etching was found to improve the bond strength of the universal adhesive to dentin when saliva contamination occurred after adhesive polymerization.[39] Rinsing with water and air drying followed by reapplication of the adhesive restored bond strength to saliva-contaminated dentin.[40] A direct comparison is again not possible due to the substrates used in the present study and the studies mentioned above (enamel vs. dentine).

It could be interesting to observe and compare the microleakage of the uncontaminated and contaminated surfaces. Therefore, the lack of control groups in the present study might be a limitation. However, this methodological approach was not preferred due to the study design and the abundant evidence in dentistry literature that showed the deleterious effects of saliva contamination during restorative procedures.[28,24,10]

Conclusions

1. Etch-and-rinse adhesive reduced microleakage of the fissure sealant applied during saliva contamination.

2. Enameloplasty reduced microleakage of the fissure sealant applied during saliva contamination.

3. Saliva contamination occurring after polymerization of the bonding agent reduced the microleakage of the fissure sealant.

4. Repeating the entire procedure after saliva contamination reduced the microleakage of the fissure sealant.

References

1.Ahovuo-Saloranta A, Forss H, Walsh T, Nordblad A, et al. Pit and fissure sealants for preventing dental decay in permanent teeth. Cochrane Database Syst Rev 2017;7:CD001830.

2.Greenwell AL, Johnsen D, DiSantis TA, Gerstenmaier J, et al. Longitudinal evaluation of caries patterns form the primary to the mixed dentition. Pediatr Dent 1990;12:278-82.

3.Memarpour M, Rafiee A, Shafiei F, Dorudizadeh T, et al. Adhesion of three types of fissure sealant in saliva-contaminated and noncontaminated conditions: an in vitro study. Eur Arch Paediatr Dent 2021;22:813-21.

4.Simonsen RJ, Neal RC. A review of the clinical application and performance of pit and fissure sealants. Aust Dent J 2011;56:45-58.

5.Memarpour M, Shafiei F, Zarean M, Razmjoei F. Sealing effectiveness of fissure sealant bonded with universal adhesive systems on saliva-contaminated and noncontaminated enamel. J Clin Exp Dent 2018;10:e1-e6.

6.Simonsen RJ. Pit and fissure sealant: review of the literature. Pediatr Dent 2002;24:393-414.

7.Feigal RJ, Hitt J, Splieth C. Retaining sealant on salivary contaminated enamel. J Am Dent Assoc 1993;124:88-97.

8.Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, et al. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res 2000;79:1850-56.

9.Hitt JC, Feigal RJ. Use of a bonding agent to reduce sealant sensitivity to moisture contamination: an in vitro study. Pediatr Dent 1992;14:41-46.

10.Borsatto MC, Corona SA, Alves AG, Chimello DT, et al. Influence of salivary contamination on marginal microleakage of pit and fissure sealants. Am J Dent 2004;17:365-67.

11.Cehreli ZC, Gungor HC. Quantitative microleakage evaluation of fissure sealants applied with or without a bonding agent: results after four-year water storage in vitro. J Adhes Dent 2008;10:379-84.

12.Botton G, Morgental CS, Scherer MM, Lenzi TL, et al. Are self-etch adhesive systems effective in the retention of occlusal sealants? A systematic review and meta-analysis. Int J Paediatr Dent 2016;26:402-11.

13.Erbas Unverdi G, Atac SA, Cehreli ZC. Effectiveness of pit and fissure sealants bonded with different adhesive systems: a prospective randomized controlled trial. Clin Oral Investig 2017;21:2235-43.

14.Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater 2001;17:430-44.

15.De Munck J, Van Landuyt K, Peumans M, Poitevin A, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005;84:118-32.

16.Burrow MF, Burrow JF, Makinson OF. Pits and fissures: etch resistance in prismless enamel walls. Aust Dent J 2001;46:258-62.

17.De Craene GP, Martens C, Dermaut R. The invasive pit-and-fissure sealing technique in pediatric dentistry: an SEM study of a preventive restoration. ASDC J Dent Child 1988;55:34-42.

18.Gungor HC, Altay N, Batirbaygil Y, Unlu N. In vitro evaluation of the effect of a surfactant-containing experimental acid gel on sealant microleakage. Quintessence Int 2002;33:679-84.

19.Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent 1999;27:523-30.

20.Hitmi L, Attal JP, Degrange M. Influence of the time-point of salivary contamination on dentin shear bond strength of 3 dentin adhesive systems. J Adhes Dent 1999;1:219-32.

21.Yazici AR, Tuncer D, Dayangac B, Ozgunaltay G, et al. The effect of saliva contamination on microleakage of an etch-and-rinse and a self-etching adhesive. J Adhes Dent 2007;9:305-09.

22.Nair P, Ilie N. The long-term consequence of salivary contamination at various stages of adhesive application and clinically feasible remedies to decontaminate. Clin Oral Investig 2020;24:4413-26.

23.Raskin A, Tassery H, D'Hoore W, Gonthier S, et al. Influence of the number of sections on reliability of in vitro microleakage evaluations. Am J Dent 2003;16:207-10.

24.Duangthip D, Lussi A. Microleakage and penetration ability of resin sealant versus bonding system when applied following contamination. Pediatr Dent 2003;25:505-11.

25.Nair P, Hickel R, Ilie N. Adverse effects of salivary contamination for adhesives in restorative dentistry. A literature review. Am J Dent 2017;30:156-64.

26.Lepri TP, Souza-Gabriel AE, Atoui JA, Palma-Dibb RG, et al. Shear bond strength of a sealant to contaminated-enamel surface: influence of erbium : yttrium-aluminum-garnet laser pretreatment. J Esthet Restor Dent 2008;20:386-92.

27.Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. J Am Dent Assoc 1985;110:329-32.

28.Borem LM, Feigal RJ. Reducing microleakage of sealants under salivary contamination: digital-image analysis evaluation. Quintessence Int 1994;25:283-89.

29.Lopes LS, Calazans FS, Hidalgo R, Buitrago LL, et al. Six-month follow-up of cervical composite restorations placed with a new universal adhesive system: A randomized clinical trial. Oper Dent 2016;41:465-80.

30.Abdalla AI, Davidson CL. Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. Am J Dent 1998;11:281-85.

31.Cardoso MV, de Almeida Neves A, Mine A, Coutinho E, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. Aust Dent J 2011;56 Suppl 1:31-44.

32.Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials 2007;28:3757-85.

33.Garcia-Godoy F, Gwinnett AJ. Penetration of acid solution and gel in occlusal fissures. J Am Dent Assoc 1987;114:809-10.

34.Torii Y, Itou K, Hikasa R, Iwata S, et al. Enamel tensile bond strength and morphology of resin-enamel interface created by acid etching system with or without moisture and self-etching priming system. J Oral Rehabil 2002;29:528-33.

35.Retief DH, Middleton JC, Jamison HC. Optimal concentration of phosphoric acid as an etching agent. Part III: Enamel wettability studies. J Prosthet Dent 1985;53:42-46.

36.Geiger SB, Gulayev S, Weiss EI. Improving fissure sealant quality: mechanical preparation and filling level. J Dent 2000;28:407-12.

37.Kermanshah H, Ghabraei S, Bitaraf T. Effect of salivary contamination during different bonding stages on shear dentin bond strength of one-step self-etch and total etch adhesive. J Dent (Tehran) 2010;7:132-38.

38.Simsek H, Yazici AR, Gungor HC. In vitro evaluation of different protocols for preventing microle-akage of fissure sealants placed following saliva contamination. J Clin Pediatr Dent 2020;44:240-48.

39.Kim J, Hong S, Choi Y, Park S. The effect of saliva decontamination procedures on dentin bond strength after universal adhesive curing. Restor Dent Endod 2015;40:299-305.

40.Santschi K, Peutzfeldt A, Lussi A, Flury S. Effect of salivary contamination and decontamination on bond strength of two one-step self-etching adhesives to dentin of primary and permanent teeth. J Adhes Dent 2015;17:51-57.