In Vitro Evaluation of Different Protocols for Preventing Microleakage of Fissure Sealants Placed Following Saliva Contamination

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Purpose: *To evaluate the effect of different enamel conditioning protocols and their re-application on the microleakage of fissure sealants placed following saliva contamination.* ***Study design:*** *The study included 156 human third molars in 16 subgroups (2X4X2) under two main groups (sealant type):* ***Group A-*** *hydrophobic resin sealant, 3M Clinpro™ Sealant;* ***Group B-*** *hydrophilic resin sealant, Ultraseal XT Hydro. Each group was then divided according to the type of surface conditioning;* ***1-*** *Er,Cr:YSSG laser etching,* ***2-*** *acid-etching,* ***3-*** *acid-etching+etch-and-rinse adhesive (Prime&Bond® One Select) and* ***4-*** *self-etching adhesive (Clearfil™ SE Bond). After contaminating the conditioned occlusal enamel surfaces with artificial saliva, fissure sealant was applied in half of the specimens* ***(a)****, whereas in the other half,* ***(b)*** *the respective surface conditioning was repeated and then fissure sealant was placed. Following thermocycling, the samples were immersed in basic fuchsin, sectioned, and dye penetration was quantitatively assessed with ImageJ. Two-way ANOVA and Bonferroni post-hoc tests were used for statistical analyses ($p<0.05$).* ***Results:*** *The least microleakage was observed in A3b and A3a, whereas B4b and B4a were the subgroups with the highest microleakage. Following saliva contamination, when surface conditioning was not re-applied, the effects of fissure sealant types and surface conditioning were significant ($p=0.005$ and $p<0.001$, respectively). However, their interaction was insignificant ($p=0.173$). When surface conditioning was re-applied after saliva contamination, the effects of type of fissure sealant and surface conditioning ($p=0.000$, for both) and their interaction ($p=0.004$) were significant.* ***Conclusions:*** *3M Clinpro™ Sealant was superior to Ultraseal XT Hydro. Re-application of Er,Cr:YSSG laser and the self-etching adhesive did not affect the microleakage of both fissure sealants. Without re-application, acid-etching+etch-and-rinse adhesive was superior to acid-etching only. However, both of them were similarly successful when they were re-applied following saliva contamination.*

Keywords: *Pit and fissure sealants, saliva contamination, microleakage, etch-and-rinse adhesive, self-etching adhesive, Er,Cr:YSSG laser*

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INTRODUCTION

Despite the decline in the prevalence of smooth surface caries, epidemiological data still show that occlusal surfaces carry most of the caries burden in children and adolescents.[1] The complex morphology of pits and fissures restrict the beneficial effect of fluorides and brushing for caries prevention. Resin-based pit-and-fissure sealants whose effectiveness are closely related to their retention and the ability to resist microleakage are the most effective means to control caries arising from these sites.[2, 3]

Etching the enamel with various concentrations of phosphoric acid has been the standard method for surface conditioning. The procedure creates microporosities that serve for the marginal integrity and retention of the sealant material by increasing its bond strength to the enamel.[3] When compared to that of conventional acid-etching, similar surface morphology changes have also been reported after laser irradiation.[4] However, saliva contamination of the conditioned enamel leads to inadequate adhesion, loss of fissure sealant, and formation of secondary caries due to microleakage.[(2, 5]

The use of a hydrophilic adhesive resin as an intermediate layer under the sealant material has been proposed particularly to overcome the consequences of saliva contamination.[2, 6] Successful results, in terms of microleakage resistance and retention, were reported in laboratory and clinical studies using this method.[6-10] The use of etch-and-rinse adhesives led to higher micromechanical bond strengths.[6, 9, 10] Self-etching adhesives, on the other hand, offer ease of use because they do not comprise separate etching, rinsing and drying steps.[11] This reduces the risk of contamination, especially in child patients difficult to cooperate. However, these types of adhesives were claimed to be unable to provide a strong bond to unground enamel as they cannot solve the aprismatic enamel layer.[12]

The effect of saliva contamination occurring at different stages of bonding with etch-and-rinse and self-etching adhesives has been evaluated.[13, 14] Yazici *et al*[14] reported that contamination with saliva before and after curing did not worsen the microleakage of a two-step etch-and-rinse or a one-step self-etching adhesive. However, Hitmi *et al*[13] have stated that the contamination at different stages had different effects on the shear bond strength of composite resin bonded with three dentin adhesives.

In recent years, sealants with reduced "moisture sensitivity" have been developed and marketed for use in cases with risk of salivary contamination. On the contrary to hydrophobic resin-based fissure sealants, these materials do not contain bisphenol-A glycidyl methacrylate (bis-GMA) monomer and are called as "hydrophilic fissure sealants".[4, 15, 16]

The objective of this *in vitro* study was to evaluate the effect of different surface conditioning protocols and their re-applications on microleakage of a hydrophobic resin-based and a hydrophilic fissure sealant following saliva contamination. Accordingly, the tested null hypotheses were as follows:

1. There is no difference between the microleakage of hydrophobic and hydrophilic fissure sealants applied following saliva contamination.
2. Type of surface conditioning does not affect the microleakage of the fissure sealants applied following saliva contamination.
3. Re-application of surface conditioning following saliva contamination does not affect the microleakage of the fissure sealants applied.

MATERIALS AND METHOD

The study protocol was approved by the human subjects ethical committee of the university where the study was carried out. Freshly extracted human third molars were collected and stored in distilled water at 4°C up to one month. The water was changed weekly to prevent bacterial growth. After surface debridement with a hand scaling instrument, 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 teeth with caries, surface cracks or developmental defects.

The study comprised 16 subgroups with a 2X4X2 study design. Two main groups were formed with respect to the fissure sealant material used: Group A–a resin-based hydrophobic sealant, 3M Clinpro™ Sealant (3M, St. Paul, Minnesota, USA) and Group B–an acrylic-based hydrophilic sealant, Ultraseal XT™ Hydro® (Ultradent, South Jordan, Utah, USA). Under each main group, eight subgroups were formed according to the surface conditioning employed (1-4) and with or without its re-application (a or b) following contamination with artificial saliva (0.4 g NaCl, 1.21 g KCl, 0.78 g $NaH_2PO_4 2H_2O$; 0.005 g $Na_2S_9H_2O$, 1 g $CO(NH_2)^2$ and 1000 ml distilled water).

The types of surface conditioning were 1- laser etching, 2- acid etching, 3- acid etching + etch-and-rinse mode of a universal adhesive (Prime&Bond® Select One; Dentsply Sirona Konstanz, Germany), 4- self-etching adhesive system (Clearfil™ SE Bond; Kuraray, Okayama, Japan). Table 1 presents the chemical composition of the fissure sealants and adhesive systems used. Manufacturers' instructions were followed during all application procedures in subgroups (Table 2).

Subgroup 1a: The occlusal surfaces were conditioned with an Erbium, Chromium: Yttrium Scandium Gallium Garnet (Er,Cr:YSGG) laser (Waterlase MD, Biolase, Irwin, California, USA) with a wavelength of 2.97 **µm**. The power output was set at 1.5 W with a repetition rate of 20 Hz and pulse duration of 140 µsec. Air and water were sprayed through the handpiece at a level of 70% water and 60% air to prevent enamel surfaces from overheating. The laser beam was delivered using a sapphire tip (600 µm in diameter and 6 mm in length) in the non-contact mode that was directed perpendicular to enamel at 1mm distance. The time of irradiation was an average of 10s. After irradiation, fissures were washed and air-dried. Occlusal enamel surfaces were contaminated with artificial saliva for 5 seconds then, washed and air-dried. The respective fissure sealant was applied and polymerized with an LED curing unit (3M Elipar S10; 3M ESPE, St. Paul, USA) with an intensity of 1200 mW/cm^2 for 40 seconds. The tip of the light source was placed on the occlusal cusps, making it possible to keep the minimum distance from the occlusal surface during polymerization.

Subgroup 1b: The occlusal surfaces were conditioned as in subgroup 1a. After contamination with artificial saliva, the teeth were washed and air-dried. Laser irradiation was repeated. The respective fissure sealant was applied and polymerized.

Subgroup 2a: The occlusal surfaces were etched with 37% phosphoric acid (I-Dental, Siauliai, Lithuania) for 30 seconds, washed and air-dried. Surfaces were then contaminated with artificial saliva, washed and air-dried. The respective fissure sealant was applied and polymerized.

Subgroup 2b: The occlusal surfaces were conditioned as in subgroup 2a. After contamination with artificial saliva, acid etching step was repeated. The teeth were washed and air-dried. The respective fissure sealant was applied and polymerized.

Subgroup 3a: The occlusal surfaces were etched with 37% phosphoric acid (I-Dental, Siauliai, Lithuania) for 30 seconds, washed and air-dried. Prime&Bond® One Select was applied and light cured. Occlusal enamel surfaces were contaminated with artificial saliva, washed and air-dried. The respective fissure sealant was applied and polymerized.

Subgroup 3b: The occlusal surfaces were conditioned as in subgroup 3a. After contamination with artificial saliva, acid etching and application of Prime&Bond® One Select was repeated. The respective fissure sealant was applied and polymerized.

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

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
Ultraseal XT® Hydro™	Ultradent, South Jordan, Utah, USA	TEGDMA, DMA, Aluminium oxide, Methacrylic acid, Titanium Dioxide, Sodium monofluorophosphate
Primer&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
Articial Saliva Solution	-	0.4 g NaCl, 1.21 g KCl, 0.78 g $NaH_2PO_42H_2O$; 0.005 g $Na_2S_9H_2O$, 1 g $CO(NH_2)^2$ and 1000 ml distilled water

TEGDMA; triethylene glycol dimethacrylate, bis-GMA; bisphenol-A glycidyl methacrylate, EDMAB; ethyl 4-dimethyl aminobenzoate, DMA; diurethane dimethacrylate, UDMA; urethane dimethacrylate, HEMA; hydroxyethyl methacrylate, PENTA; phosphonated penta-acrylate ester, MDP;10-methacyloxidesyl dihydrogen, NaCl; sodium chloride, KCl; potassium chloride.

Subgroup 4a: The occlusal surfaces were conditioned with the primer of a self-etching adhesive, Clearfil™ SE Bond, waited for 20 sec, air blowed gently and followed by the application of the bond of Clearfil™ SE Bond that was light-cured for 10 sec. After contamination with artificial saliva, the surfaces were washed and dried. The respective fissure sealant was applied and polymerized.

Subgroup 4b: The same procedures were followed as in subgroup 4a. After contamination with artificial saliva, the application of Clearfil™ SE Bond was repeated. The respective fissure sealant was applied and polymerized.

Following storage in distilled water at 37° C for one week, all specimens were subjected to thermocycling for 1000 cycles, in 5 ± 2°C to 55 ± 2°C with a dwell time of 15s and a transfer time of 10 seconds. The apices were sealed with sticky wax, and the samples were coated with two consecutive layers of nail varnish to within 1 mm of the sealant margins. The specimens 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. After the samples were embedded in chemically activated acrylic resin (Integra, BG Dental, Turkey), four sections of 0.5 mm thickness were obtained from each tooth using a slow-speed, water-cooled diamond saw (Micracut 201, Metkon, Bursa, Turkey). A digital photograph of each section was taken at 20X under a stereomicroscope (Olympus SZ61, Tokyo, Japan), and the images were transferred to a Macintosh PowerPC workstation. An open-source image analysis software (ImageJ for MacOSX; V.1.34, National Institutes of Health; Bethesda, MD, USA) was used to measure the extent of buccal and lingual dye penetration along the enamel/fissure sealant interface (in mm). One calibrated operator, blinded to treatment groups, made the measurements. 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).

The data were analyzed using two-way ANOVA for application and another two-way ANOVA for the re-application of surface conditioning. Multiple comparisons were made using Bonferroni post-hoc test. For all statistical analyses, SPSS 26.0 (SPSS, Chicago, IL, USA) was used. The level of significance was set as $\alpha = 0.05$.

RESULTS

The present study was carried out on a total of 624 sections obtained from 156 teeth. Seventeen sections were not used since they were not suitable for measurement. Hence, the measurements were completed on a total of 607 sections. The microleakage values obtained in the study were presented in Table 3.

The overall mean microleakage values of Group A (3M Clinpro™ Sealant) were significantly less than those of Group B (Ultraseal XT® Hydro™) ($p<0.05$). As regards Group A, the least microleakage was observed in A3a when surface conditioning was not re-applied following saliva contamination. It was followed by A2a<A4a<A1a ($p<0.05$). For Group B, the least microleakage was found in B3a. It was followed by B2a<B1a<B4a ($p<0.05$). The type of fissure sealant and surface conditioning were significantly effective ($p=0.005$ and $p<0.001$, respectively). However, their interaction was insignificant ($p=0.173$).

When surface conditioning was re-applied following saliva contamination, the least microleakage was observed in A3b of Group A. It was followed by A2b ($p>0.05$), and A4b<A1b ($p<0.05$). For Group B, the least microleakage was found in B3b ($p<0.05$). It was followed by B2b<B1b=B4b ($p<0.05$). When surface conditioning was re-applied, the effects of type of fissure sealant and surface conditioning ($p=0.000$, for both) and their interaction ($p=0.004$) were all significant.

Multiple comparisons for interactions between types of surface conditioning were presented in Table 4. Analyses revealed that there was no significant difference between no re-application and re-application of laser etching and Clearfil™ SE Bond ($p>0.05$ for both). The interaction between acid etching and acid etching + Prime&Bond®

Table 2. Detailed application protocol of the study materials

Groups	Type of Surface Conditioning	Contaminate with	After contamination	Subgroups	Application protocol
Group A 3M Clinpro™ Sealant	• Laser etching	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	A1a	• No re-conditioning
				A1b	• Laser-etch
	• Etch with 37% phosphoric acid for 30 s. • Wash with water spray for 30 s • Air-dry for 10 s	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	A2a	• No re-conditioning
				A2b	• Etch with 37% phosphoric for 30 s. • Wash with water spray for 30 s • Air-dry for 10 s
	• Etch with 37% phosphoric acid for 30 s. • Wash 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	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	A3a	• No re-conditioning
				A3b	• Etch with 37% phosphoric for 30 s. • Wash 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 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	Artificial Saliva (5 s)	Wash with water spray for 30 s Air-dry for 10 s	A4a	• No re-conditioning
				A4b	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
Group B **Ultraseal XT® Hydro™**	• Laser-etch	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	B1a	• No re-conditioning
				B1b	• Laser-etch
	• Etch with 37% phosphoric acid for 30 s. • Wash with water spray for 30 s • Air-dry for 10 s	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	B2a	• No re-conditioning
				B2b	• Etch with 37% phosphoric acid for 30 s. • Wash with water spray for 30 s • Air-dry for 10 s
	• Etch with 37% phosphoric acid for 30 s. • Wash 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	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	B3a	• No re-conditioning
				B3b	• Etch with 37% phosphoric acid for 30 s. • Wash 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 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	Artificial Saliva (5 s)	• Wash with water spray for 30 s • Air-dry for 10 s	B4a	• No re-conditioning
				B4b	• 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

Figure 1. Scoring system for the evaluation of microleakage (modified from Duangthip and Lussi [19]). 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.

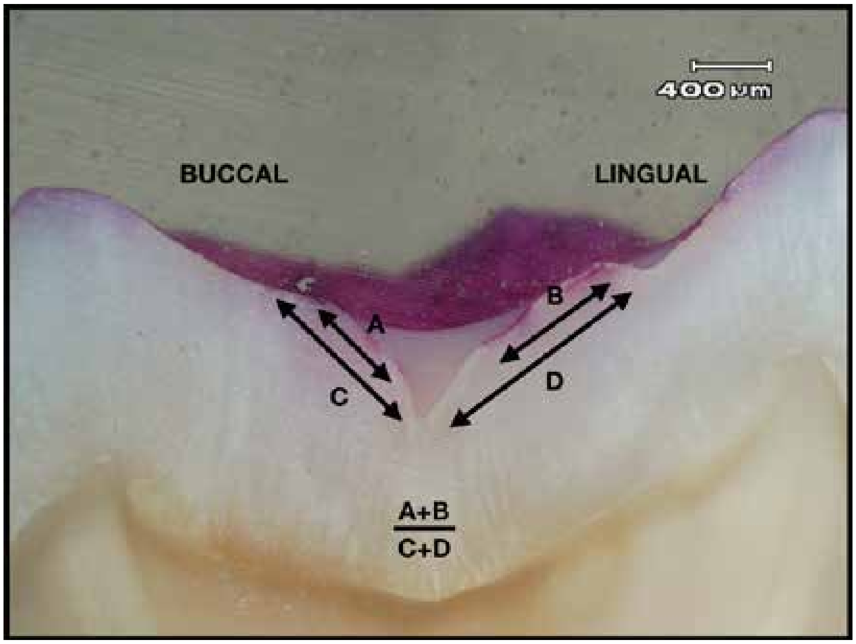

Figure 2. Stereomicroscopic views of microleakage obtained in subgroups.

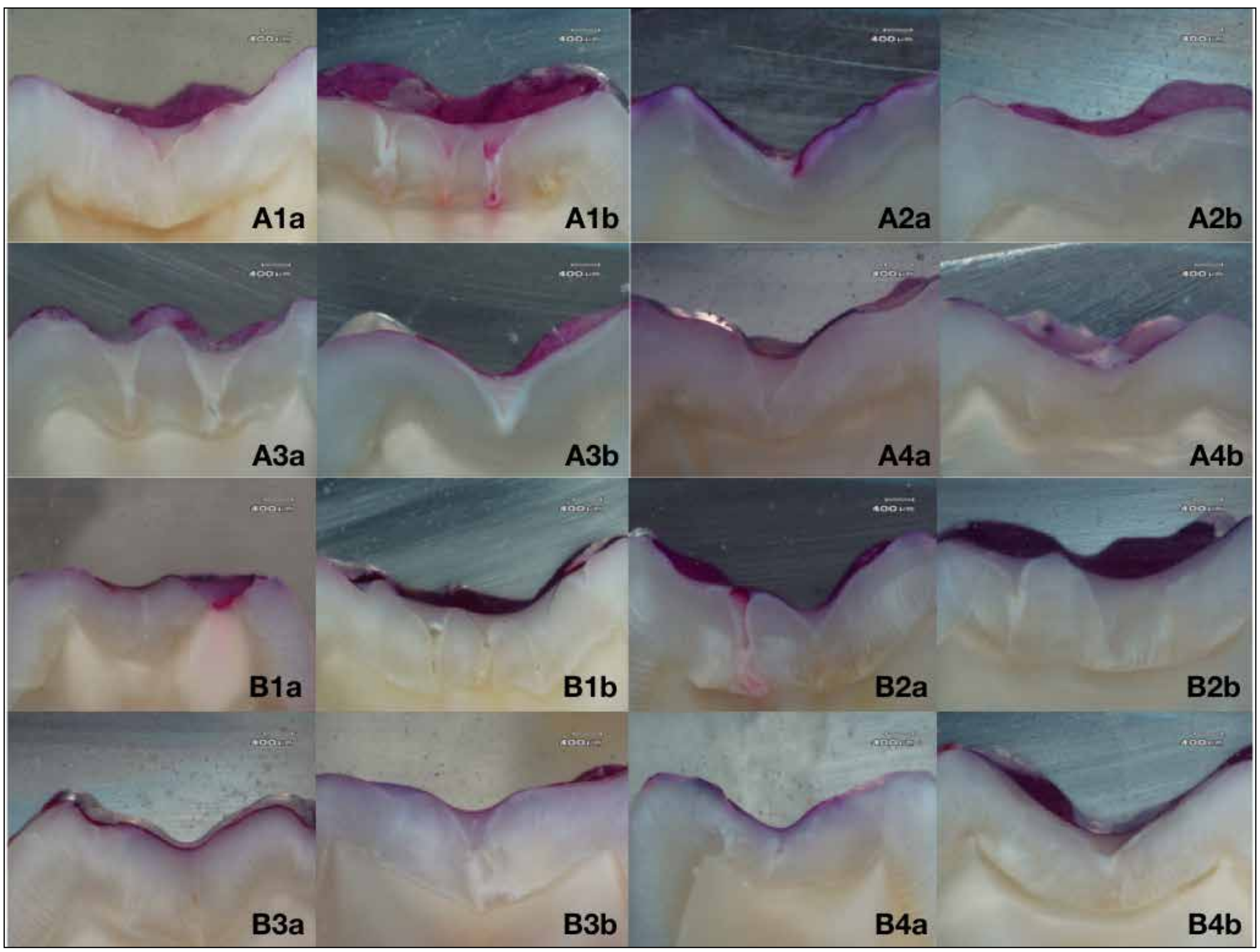

Table 3: Mean and standard deviation of microleakage for two types of fissure sealant and four types of conditioning

Dependent Variable			No Re-application of Surface Conditioning			Re-application of Surface Conditioning		
Type of Fissure Sealant	Type of Surface Conditioning	N	Subgroup	Mean	Std. Deviation	Subgroup	Mean	Std. Deviation
Group A 3M Clinpro™ Sealant	Laser etching	9	A1a	0,6434	0,1616	A1b	0,6009	0,2056
	Acid etching	10	A2a	0,4129	0,1548	A2b	0,2255	0,0950
	Acid etching + Prime&Bond Select One	10	A3a	0,1587	0,0880	A3b	0,1099	0,1074
	Clearfil SE Bond	10	A4a	0,5501	0,2359	A4b	0,5151	0,0842
Group B Ultraseal XT™ Hydro®	Laser etching	9	B1a	0,7454	0,1719	B1b	0,7211	0,1144
	Acid etching	10	B2a	0,5054	0,3261	B2b	0,2442	0,2421
	Acid etching + Prime&Bond Select One	10	B3a	0,1767	0,1181	B3b	0,1291	0,0974
	Clearfil SE Bond	10	B4a	0,8250	0,1084	B4b	0,8381	0,1340
Total	Laser etching	18		0,6944	0,1701		0,6610	0,1728
	Acid etching	20		0,4592	0,2529		0,2349	0,1792
	Acid etching + Prime&Bond Select One	20		0,1677	0,1018		0,1195	0,1003
	Clearfil SE Bond	20		0,6876	0,2276		0,6766	0,1982

Note: When surface conditioning was not re-applied, the effects of fissure sealant types and surface conditioning were significant (p=0.005 and p<0.001, respectively). However, their interaction was insignificant (p=0.173). When surface conditioning was re-applied after saliva contamination, the effects of type of fissure sealant and surface conditioning (p=0.000, for both) and their interaction (p=0.004) were all significant.

Table 4: P value for Bonferroni post-hoc test (multiple comparisons)

Surface Conditioning	No Re-application of Surface Conditioning	Re-application of Surface Conditioning
Type of interaction	P	P
Laser etching and Acid etching	0.001	0.000
Laser etching and Acid etching + Prime&Bond One Select	0.000	0.000
Laser etching and Clearfil SE Bond	1.000	1.000
Acid etching and Acid etching + Prime&Bond One Select	0.000	0.084
Acid etching and Clearfil SE Bond	0.001	0.000
Acid etching + Prime&Bond One Select and Clearfil SE Bond	0.000	0.000

One Select was significant for no re-application (p<0.05). However, their re-application following saliva contamination did not result in significance (p>0.05). Except for these, all interactions for no re-application and re-application were significant (p<0.05).

DISCUSSION

Saliva contamination is frequently observed in fissure sealant applications if rubber-dam is not used. It is especially encountered either during or following the rinse of the etchant. This is a critical step where contamination, for as short as one second, has been shown to result in the formation of a surface coating that cannot be removed effectively by rinsing.[17] The condition significantly risks the retention and sealing effectiveness of the fissure sealant.[9] The present study evaluated the re-application of different surface conditioning types to eliminate the effects of saliva contamination.

Microleakage tests are one of the methods to evaluate the sealing performance of adhesive systems.[18] Among different methods employed, 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.[18] This technique was combined with digital image analysis in order to obtain quantitative results instead of a conventional subjective scoring.[9] The relative merit of this objective approach, compared to a subjective scoring system, was to discard the need for scoring by separate evaluators and for consensus scoring in borderline cases, as well as statistical procedures with regard to interexaminer reliability.[19]

The use of "moisture tolerant" or "hydrophilic sealants" has been suggested to overcome the challenges of saliva contamination when a rubber dam is not in use.[15] One of the materials of the present study, Ultraseal XT® Hydro™, is a light-cured and acrylic-based fissure sealant. It has been reported that this hydrophilic material removes moisture from pits and fissures, thereby eliminating moisture-related failure in hydrophobic fissure sealants.(4) The microleakage resistance of Ultraseal XT® Hydro™ has been evaluated on extracted human molars.[4, 16] Acid-etching or Er:YAG laser irradiation with acid-etching was preferred in the first study. The authors concluded that laser conditioning significantly reduced microleakage of Ultraseal XT® Hydro™.[4] In the second study, acid-etching, Er:YAG laser irradiation or laser irradiation + acid-etching have been chosen as surface conditioning methods.(16) The authors reported no significant differences in microleakage between the acid-etched and Er:YAG laser-irradiated groups. In addition, the teeth treated with laser irradiation + acid-etching, Ultraseal XT® Hydro™ demonstrated significantly lower microleakage.[16] However, both studies did not have control groups (i.e., another fissure sealant material). Gawali *et al* [20] also compared microleakage of Ultraseal XT® Hydro™ to that of Fissurit F (a resin-based hydrophobic fissure sealant) on primary molars following saliva contamination. While Ultraseal XT® Hydro™ was found to be more successful in preventing microleakage, Fissurit F was superior in terms of penetration ability.

In the present study, Ultraseal XT® Hydro™ significantly showed more microleakage than 3M Clinpro™ Sealant. The results of the present study are in line with those of studies that reported a significant increase in microleakage with the use of hydrophilic fissure sealants applied after saliva contamination.[21, 22] This finding was similar for both no re-application and re-application of surface conditioning after saliva contamination. Hence, the first null hypothesis of the study was rejected.

The second null hypothesis was also rejected since the study results showed that the type of surface conditioning affects the microleakage of the sealant. In both sealant group where surface conditioning was not repeated following saliva contamination, the use of Er,Cr:YSGG laser and Clearfil SE™ Bond led to higher levels of microleakage. A similar finding has been reported by Lupi-Pegurier *et al*[23] Their study showed stated that the microleakage values of groups in which an Er:YAG laser was used alone before fissure sealing were higher than those of acid-etching and laser combination. In other studies, significantly higher microleakage values were also observed with the use of Er:YAG laser in fissure sealant applications.[24, 25]

Due to the ring of aprismatic enamel surrounding the entrance and walls of fissures, the occlusal fissures are considered resistant to etching.[26] The limited depth of decalcification on the core of the enamel prism due to total inactivation of the acid when it comes into contact with the enamel surface[12] results in a thin layer between the resin composite and the thin, lamina-like resin extensions.[27] Aprismatic enamel is also known to be less conducive to bonding by self-etching adhesives.[28] Methacrylated phosphoric acid esters (also present in the tested self-etching adhesive) form more shallow etching patterns than those observed with phosphoric acid etching.[11] Additionally, the presence of dissolved calcium phosphates that cannot be removed while using self-etching adhesive systems (since it is not rinsed) may result in lower resistance to thermomechanical stress, and development of marginal openings of the fissure sealing.[12, 28, 29].

Clearfil SE™ Bond also contains 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. HEMA is included to offer strength to cross-linking formed from the monomeric matrix. It has been reported that 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.[30] These may help to explain the related findings obtained in the present study.[31]

The findings of the present study indicated that acid etching + Prime&Bond® One Select was the most successful subgroup where surface conditioning was not repeated following saliva contamination. Prime&Bond® One Select is an acetone-based adhesive system which has nano-fillers, a cross-linked molecule, T-resin and D-resin, a small molecule of fluid. These resins and nano-fillers have been reported to increase the adhesion to the acid-etched dentin.[32] It should be noted that the surfaces were contaminated with artificial saliva after the polymerization of the adhesive. The possible effects of saliva contamination on the microleakage of different types of adhesive systems have been evaluated.[14] When the tooth surface is contaminated with saliva after application of the adhesive, but before polymerization, the degree of conversion may be affected.[33] 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 alteration of the bond strength.[33] On the other hand, if contamination occurs after 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.[33] Hitmi *et al*[13] evaluated the changes in shear bond strength of etch-and-rinse and self-etching adhesives. They found that the saliva contamination occurring before the application of etch-and-rinse adhesive resulted in decreased bond strength values. They also stated that the bond strength values of both etch-and-rinse and self-etching adhesives significantly decreased when saliva contamination occurred after the application of the bonding agents. The researchers related their findings to the oxygen and water contained in saliva, which prevented the polymerization of the bonding agents. Prime&Bond® One Select is a relatively new adhesive system which warrants further studies to be carried out on enamel surfaces.

As regards the re-application of surface conditioning, both acid-etching and acid-etching + Prime&Bond® One Select were successful. Their differences were insignificant. However, in other subgroups where Er,Cr:YSGG laser and Clearfil SE™ Bond were used, their re-application was found to be unuseful. Hence, the third null hypothesis of the study was partially accepted.

Acid etching is an essential step for bonding resin-based materials to the enamel.[3] Its use with etch-and-rinse adhesives has been reported to reduce microleakage and increase the clinical success of fissure sealants that were applied following saliva contamination.[5, 7, 31, 34] The tested adhesive, Prime&-Bond® One Select, is a universal adhesive which can be used in etch-and-rinse, selective etch and self-etching modes.[32] Its solvent is acetone, which is a "water chaser" and its boiling point increases and that of water decreases and when an acetone-based primer comes in contact with the moistened surface. Acetone and water then evaporate, leaving behind the resin.[35] The finding mentioned above is interesting in that, even when used alone, re-application of acid etching is as successful as re-application an "etch and bond" procedure under salivary contamination conditions. It also provides strong support for the recommendation to repeat acid etching in cases of contamination during fissure sealant applications.[36]

The use of artificial saliva is a limitation of the present study. Nair *et al*[33] have questioned the use of artificial saliva, although these formulations try to have a composition similar to that of natural saliva, which comprises several hydrolytic enzymes among other organic constituents. These enzymes react with the tooth structure through different biochemical processes, which could modify the surface of the tooth structure and also compromise the material bond strength.[37] Another limitation could be the lack of control groups. This methodological approach was not preferred due to the study design and abundant evidence in dentistry literature showing the deleterious effects of saliva contamination on restorative procedures.[8, 19, 34]

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions could be drawn.

1. The resin-based hydrophobic fissure sealant (3M Clinpro™ Sealant) was superior to the moisture-tolerant fissure sealant (Ultraseal XT™ Hydro®) in terms of resisting microleakage under salivary contamination.
2. Re-application of Er,Cr:YSSG and Clearfil™ SE Bond did not affect the microleakage of both fissure sealants.
3. Without re-application, acid-etching+etch-and-rinse adhesive was superior to acid-etching only. However, both of them were similarly successful when they were re-applied following saliva contamination.

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REFERENCES

1. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res; 38:182-191. 2004.

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

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

4. Guclu ZA, Donmez N, Hurt AP, Coleman NJ. Characterisation and microleakage of a new hydrophilic fissure sealant–UltraSeal XT(R) hydro. J Appl Oral Sci; 24:344-351. 2016.

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

6. Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res; 79:1850-1856. 2000.

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

8. Borsatto MC, Corona SA, Alves AG, Chimello DT, Catirse AB, Palma-Dibb RG. Influence of salivary contamination on marginal microleakage of pit and fissure sealants. Am J Dent; 17:365-367. 2004.

9. 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; 10:379-384. 2008.

10. McCafferty J, O’Connell AC. A randomised clinical trial on the use of intermediate bonding on the retention of fissure sealants in children. Int J Paediatr Dent; 26:110-115. 2015.

11. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater; 17:430-444. 2001.

12. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res; 84:118-132. 2005.

13. 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; 1:219-232. 1999.

14. Yazici AR, Tuncer D, Dayangac B, Ozgunaltay G, Onen A. The effect of saliva contamination on microleakage of an etch-and-rinse and a self-etching adhesive. J Adhes Dent; 9:305-309. 2007.

15. Khatri SG, Samuel SR, Acharya S, Patil S, Madan K. Retention of moisture-tolerant and conventional resin-based sealant in six- to nine-year-old children. Pediatr Dent; 37:366-370. 2015.

16. Guclu ZA, Donmez N, Tuzuner T, Odabas ME, Hurt AP, Coleman NJ. The impact of Er:YAG laser enamel conditioning on the microleakage of a new hydrophilic sealant—UltraSeal XT hydro. Lasers Med Sci; 31:705-711. 2016.

17. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. J Am Dent Assoc; 110:329-332. 1985.

18. Raskin A, Tassery H, D’Hoore W, Gonthier S, Vreven J, Degrange M, et al. Influence of the number of sections on reliability of in vitro microleakage evaluations. Am J Dent; 16:207-210. 2003.

19. Duangthip D, Lussi A. Microleakage and penetration ability of resin sealant versus bonding system when applied following contamination. Pediatr Dent; 25:505-511. 2003.

20. Gawali PN, Chaugule VB, Panse AM. Comparison of microleakage and penetration depth between hydrophilic and hydrophobic sealants in primary second molar. Int J Clin Paediatr Dent; 9:291-295. 2016.

21. Bagherian A, Ahmadkhani M, Sheikhfathollahi M, Bahramabadinejad R. Microbial microleakage assessment of a new hydrophilic fissure sealant: a laboratory study. Pediatr Dent; 35:194-198. 2013.

22. Khogli AE, Cauwels R, Vercruysse C, Verbeeck R, Martens LC. Microleakage and penetration of a hydrophilic sealant and a conventional resin-based sealant as a function of preparation techniques: a laboratory study. Int J Paediatr Dent; 23:13-22. 2013.

23. Lupi-Pegurier L, Bertrand MF, Muller-Bolla M, Rocca JP, Bolla M. Comparative study of microleakage of a pit and fissure sealant placed after preparation by Er:YAG laser in permanent molars. J Dent Child (Chic); 70:134-138. 2003.

24. Francescut P, Lussi A. Performance of a conventional sealant and a flowable composite on minimally invasive prepared fissures. Oper Dent; 31:543-550. 2006.

25. Ciucchi P, Neuhaus KW, Emerich M, Peutzfeldt A, Lussi A. Evaluation of different types of enamel conditioning before application of a fissure sealant. Lasers Med Sci; 30:1-9. 2015.

26. Burrow MF, Burrow JF, Makinson OF. Pits and fissures: etch resistance in prismless enamel walls. Aust Dent J; 46:258-262. 2001.

27. Torii Y, Itou K, Hikasa R, Iwata S, Nishitani Y. 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; 29:528-533. 2002.

28. Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent; 27:523-530. 1999.

29. Hannig M, Grafe A, Atalay S, Bott B. Microleakage and SEM evaluation of fissure sealants placed by use of self-etching priming agents. J Dent; 32:75-81. 2004.

30. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials; 28:3757-3785. 2007.

31. Bagherian A, Sarraf Shirazi A, Sadeghi R. Adhesive systems under fissure sealants: yes or no?: A systematic review and meta-analysis. J Am Dent Assoc; 147:446-456. 2016.

32. Lopes LS, Calazans FS, Hidalgo R, Buitrago LL, Gutierrez F, Reis A, et al. Six-month follow-up of cervical composite restorations placed with a new universal adhesive system: A randomized clinical trial. Oper Dent; 41:465-480. 2016.

33. Nair P, Hickel R, Ilie N. Adverse effects of salivary contamination for adhesives in restorative dentistry. A literature review. Am J Dent; 30:156-164. 2017.

34. Borem LM, Feigal RJ. Reducing microleakage of sealants under salivary contamination: Digital-image analysis evaluation. Quintessence Int; 25:283-289. 1994.

35. Abdalla AI, Davidson CL. Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. Am J Dent; 11:281-285. 1998.

36. Hebling J, Feigal RJ. Use of one-bottle adhesive as an intermediate bonding layer to reduce sealant microleakage on saliva-contaminated enamel. Am J Dent; 13:187-191. 2000.

37. Finer Y, Santerre JP. Salivary esterase activity and its association with the biodegradation of dental composites. J Dent Res; 83:22-26. 2004