Bond Strength of a Pit-and-Fissure Sealant Associated to Etch-and-Rinse and Self-Etching Adhesive Systems to Saliva-Contaminated Enamel: Individual vs. Simultaneous Light Curing

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This study evaluated *in vitro* the shear bond strength (SBS) of a resin-based pit-and-fissure sealant [Fluroshield (F), Dentsply/Caulk] associated with either an etch-and-rinse [Adper Single Bond 2 (SB), 3M/ESPE] or a self-etching adhesive system [Clearfil S3 Bond (S3), Kuraray Co., Ltd.] to saliva-contaminated enamel, comparing two curing protocols: individual light curing of the adhesive system and the sealant or simultaneous curing of both materials. Mesial and distal enamel surfaces from 45 sound third molars were randomly assigned to 6 groups (n=15), according to the bonding technique: I - F was applied to 37% phosphoric acid etched enamel. The other groups were contaminated with fresh human saliva (0.01 mL; 10 s) after acid etching: II - SB and F were light cured separately; III - SB and F were light cured together; IV - S3 and F were light cured separately; V - S3 and F were light cured simultaneously; VI - F was applied to saliva-contaminated, acid-etched enamel without an intermediate bonding agent layer. SBS was tested to failure in a universal testing machine at 0.5 mm/min. Data were analyzed by one-way ANOVA and Fisher's test (α=0.05).The debonded specimens were examined with a stereomicroscope to assess the failure modes. Three representative specimens from each group were observed under scanning electron microscopy for a qualitative analysis. Mean SBS in MPa were: I-12.28 (±4.29); II-8.57 (±3.19); III-7.97 (±2.16); IV-12.56 (±3.11); V-11.45 (±3.77); and VI-7.47 (±1.99). In conclusion, individual or simultaneous curing of the intermediate bonding agent layer and the resin sealant did not seem to affect bond strength to saliva-contaminated enamel. S3/F presented significantly higher SBS than the that of the groups treated with SB etch-and-rinse adhesive system and similar SBS to that of the control group, in which the sealant was applied under ideal dry, noncontaminated conditions.

Key Words: pit-and-fissure sealant, shear bond strength, salivary contamination, adhesive system, light curing.

INTRODUCTION

According to the contemporary dental principles, as often as possible, noninvasive strategies should preferably be instituted rather than invasive healing treatments (1). Efforts have been focused on reducing caries risk by stimulating the adoption of preventive measures and highlighting the relevance of a partnership approach between patients and dentists for ultimate success in caries control. Caries risk assessment is also an essential step to provide an individual-based, comprehensive treatment planning.

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The high susceptibility of pits and fissures to carious attack and the rapid onset of the disease at these sites soon after tooth eruption are reported by several studies. In this context, treating caries-susceptible pits and fissures with resin sealants has been considered an outstanding adjunctive resource to oral health care strategies and fluoride therapy to decrease occlusal caries initiation and/or progression (2). Nevertheless, the preventive benefits of such treatment rely directly upon the ability of the sealing material to thoroughly fill pits, fissures and/or anatomical defects, and remain completely intact and bonded to the enamel surface for a lifetime, thus preventing caries from developing underneath the sealant restoration (3). Limited use of sealants has been attributed to lack of confidence in their adhesion to enamel and to the difficulty in achieving adequate salivary control and dry field isolation (4).

Recently, there has been a shift with respect to sealant therapy indication. It has been advocated that, for sealants to be effective, they must be placed in children who are at high risk for occlusal caries and should not be applied routinely throughout a low-risk dental population (5). In high-caries-risk patients, the most appropriate moment for placement of occlusal sealants is soon after the eruption of the permanent molars. Newly erupted teeth are far less mineralized than those exposed to oral environment for years and are hence more susceptible to acid attack. Paradoxically, the possibility of failure increases for sealants placed shortly after tooth eruption, when the distal marginal ridge has just cleared in soft tissue, which leaves the occlusal surface at risk for moisture and salivary contamination during the sealing procedure (5,6). Saliva and moisture contamination of etched enamel before sealant placement is the most common reason for sealant failure and loss because the microporosities produced by the acid etchant on enamel become partially occluded, thus preventing optimal resin tag formation and undermining sealant bonding (7).

The benefits of adding a bonding agent layer between the etched enamel and the sealant in order to increase the bond strength in case of moisture and salivary contamination have been demonstrated (8-10). Other studies have found that the use of bonding agents beneath sealants placed on saliva-contaminated enamel can reduce microleakage (11), enhance resin flow into fissures and improve short-term clinical success (8,9). In a previous study of our research group, individual or simultaneous curing of an etch-and-rinse adhesive system and sealant did not affect the bond strength to enamel after salivary contamination (10). However, complete penetration of the etchant into the fissures should occur for a durable retention of the sealant to enamel. In a previous study (12), none of the tested commercially available etchants was able to penetrate farther than 17% of the total fissure depth in a fissure model. Therefore, the use of self-etching adhesive systems may be a valid and promising alternative to acid etching with phosphoric acid. These new self-etching adhesive systems are user-friendly by dental community and have been developed to simplify the bonding procedures and reduce the adhesive technique sensitivity since the enamel/dentin acid etching, rinsing and drying steps are eliminated (13). Having less operative steps and a shorter chairtime is particularly interesting when treating pediatric patients.

The literature is still scarce in studies investigating the use of self-etching adhesives systems prior to sealant placement, and the existing works have shown controversial results (9,14,15). In addition, to the best of our knowledge, no study has yet evaluated the association of this adhesive protocol under conditions of salivary contamination and testing the influence of the light-curing technique. Therefore, the purpose of this study was to evaluate *in vitro* the shear bond strength (SBS) of a resin-based pit-and-fissure sealant associated with either an etch-and-rinse or a self-etching adhesive system under salivary contamination conditions, comparing two curing protocols: individual light-curing of the intermediate bonding agent layer and the resin sealant (2 cures) or simultaneous light-curing of both materials together (1 cure).

MATERIAL AND METHODS

Freshly extracted sound human third molars were hand scaled and cleaned with water/pumice slurry in rotating bristle brushes to remove calculus and surface-adhered debris, and were examined under a ×20 magnifier to discard those with structural defects. Forty-five teeth were selected for the study and stored in 0.9% saline with 0.4% sodium azide at 4°C. Prior to use, the teeth were washed thoroughly in running water to eliminate storage solution traces, the roots were removed 3 mm below the cementoenamel junction and the crowns were embedded in polyester resin using polyvinyl chloride rings (2.1 cm diameter and 1.1 cm height). After resin polymerization, the rings were discarded and the mesial and distal enamel surfaces (n=90) were ground wet with #320- to #400-grit silicon carbide (SiC) papers (Buehler Ltd., Lake Bluff, IL, USA) in a low-speed polishing machine (Politriz DP-9U2; Struers, A/S, Copenhagen, Denmark). The specimens were then hand polished with wet #600-grit SiC paper to obtain flat, smooth test surfaces that were cleaned by rubber cup/pumice prophylaxis for 10 s. For standardization, the bonding sites were demarcated by attaching a piece of insulating tape with a 3-mm-diameter central hole on each surface. The 90 test surfaces were randomly assigned to 6 groups (n=15), according to the bonding/curing protocol adopted. The following materials were tested under different experimental conditions: a filled resin-based pit-and-fissure sealant [Fluroshield (F), Dentsply/Caulk, Milford, DE, USA)], a single-bottle etch-and-rinse adhesive system [Adper Single Bond 2 (SB), 3M/ESPE, St. Paul, MN, USA] and a self-etching adhesive system [Clearfil S3 Bond (S3), Kuraray Co., Ltd., Osaka, Japan]. All materials were used according to manufacturers' instructions.

Group I (control - dry, noncontaminated enamel) - the enamel surface was etched with a 35% phosphoric acid gel (Scotchbond etchant, 3M/ESPE) for 15 s, rinsed thoroughly for 15 s, dried with a mild, oil-free air stream to obtain a uniformly white, dull, chalk-like appearance and Fluroshield was applied. Enamel acid etching was performed in the same way in the groups in which the etch-and-rinse adhesive was used. In groups II to VI, before adhesive system application, the etched enamel bonding sites were contaminated for 10 s with 0.01 mL of fresh human whole saliva, collected from a same donor, using a micropipette. The contaminated enamel was blot dried with absorbent paper for 5 s. Fresh human saliva was used to simulate as close as possible the contamination occurred under clinical conditions. The time of 10 s was established to simulate a salivary contamination that is not perceived during the operative procedures. Group II - Adper Single Bond 2 was applied to the acid etched enamel bonding site in a uniform layer, slightly thinned with a mild, oil-free air stream and light-cured for 10 s with a visible light curing unit (XL 3000; 3M/ESPE) with a 450 mW/cm^2 output power. Next, Fluroshield sealant was applied and light-cured for 20 s; Group III - Adper Single Bond 2 was applied, gently air thinned without light curing, and Fluroshield was immediately applied over the bonding agent layer and both materials were light-cured together in one curing cycle of 20 s; Group IV - Clearfil S3 Bond was applied to the enamel bonding site in a uniform layer, gently air thinned and light-cured for 10 s. Next, Fluroshield sealant was applied and light-cured for 20 s; Group V - Clearfil S3 Bond was applied in a uniform layer, gently air thinned without light curing, and Fluroshield was immediately applied over the bonding agent layer and both materials were light-cured together in one curing cycle of 20 s; Group VI - Fluroshield was applied directly to the acid-etched enamel bonding site after salivary contamination and light-cured for 20 s.

In all groups, the adhesive systems and the sealant were carefully applied onto the delimited enamel surface with disposable microbrush tips (Microbrush Corporation, Orlando, FL, USA) to avoid excess and pooling of material along the edges of the insulating tape, which could compromise tension distribution during the test and hence the validity of results. Once the bonding protocols were completed, the specimens were individually fixed in a metallic clamping device that secured the test dentin surface parallel to a flat base. A split bisected polytetrafluoroethylene jig was positioned on the tooth/resin block, providing a cylindrical cavity with 4 mm in height and 3 mm in diameter that was coincident with the demarcated enamel bonding site. Sealant was inserted into the jig in increments, each one polymerized for 20 s. As the cavity was completely filled, the specimen was removed from the clamping device, and the jig was opened and separated, leaving a sealant cylinder (4 mm x 3 mm) adhered to the enamel surface.

After 24-h storage in distilled water at 37^{o}C, SBS was tested to failure using a knife-edge blade in a universal testing machine (Model MEM 2000, EMIC Ltda, São José dos Pinhais, PR, Brazil) running at a crosshead speed of 0.5 mm/min with a 50 kgf load cell. Mean SBS in MPa and standard deviation were calculated and data were analyzed statistically by one-way ANOVA. Fisher's exact test was used for multiple comparisons at 5% significance level. The debonded specimens were observed with a ×40 stereomicroscope to assess the failure modes, which were classified as adhesive, cohesive or mixed. Three specimens representative from each group were observed under scanning electron microscopy (SEM) for a qualitative analysis. All examinations were done by a single examiner blinded to the groups to which the specimens belonged.

RESULTS

Mean SBS and standard deviations for saliva-contaminated and non-contaminated groups are presented in Table 1.

Bond strength to saliva-contaminated enamel were remarkably lower ($p<0.05$) than those recorded under dry, noncontaminated conditions, mainly in group VI, in which the sealant was applied to saliva-contaminated acid-etched enamel without an intermediate bonding agent layer. This group was statistically similar ($p>0.05$) to groups II and III, in which a layer of an etch-and-rinse adhesive system was applied to the saliva-contaminated enamel underneath the sealant using either an individual (2 cures) or a simultaneous (1 cure) curing protocol.

No statistically significant difference ($p>0.05$) was observed among groups I, IV and V, which means that, regardless of the curing protocol, sealant association with the self-etching adhesive system under salivary contamination produced SBS similar to that of the control group (noncontaminated etched enamel).

The failure pattern on the debonded surfaces shows that most specimens in groups IV and V (40%) presented cohesive failures between the dental substrate and the adhesive layer, while adhesive failures were most frequent in groups I, II, III and VI. These types of failure are consistent with the SBS results, since a direct relationship was observed between the increase of bond strength and the occurrence of cohesive failures.

SEM micrographs representative of the three types of failure modes are illustrated in Figure 1.

Table 1. Mean shear bond strength (in MPa) to noncontaminated and saliva-contaminated enamel.

Groups	Means (SD)
I - F applied to noncontaminated enamel	12.28 (4.29)a
II - Saliva contamination + SB and F (2 cures)	8.57 (3.19)b
III - Saliva contamination + SB and F (1 cure)	7.97 (2.16)b
IV - Saliva contamination + S3 and F (2 cures)	12.56 (3.11)a
V - Saliva contamination + S3 and F (1 cure)	11.45 (3.77)a
VI - F applied to saliva-contaminated enamel	7.47 (1.99)b

Different letters indicate statistically significant difference at 5%.

DISCUSSION

The retention of resin sealants is a micromechanical process established by the infiltration and further polymerization of the sealant into the microporosity network created by the acid etchant on enamel surface. Because of the high enamel reactivity induced by acid etching, even minute exposures to saliva, as brief as 1 s, are reported to be enough to create a pellicle that partially occludes the micropores leading to an ultrastructural alteration of etched enamel and precluding the formation of the resin tags responsible for mechanical adhesion (7).

Unnoticed salivary contamination or presence of moisture in the operative field is a frequent occurrence when adequate rubber dam isolation is not achieved for sealant placement, which decreases dramatically the bond strength between the sealant and the contaminated surface, and might cause partial or total loss of the sealant restoration within a short time (4). In the present investigation, the SBS to saliva-contaminated enamel was markedly lower than that that recorded under dry, noncontaminated conditions. This study compared 2 curing protocols (individual *vs* simultaneous light curing of the intermediate bonding agent layer and resin sealant) under dry and contamination conditions, the latter being simulated by contamination of specimens with saliva from a volunteer donor.

Over the last decades, the application of bonding agent underneath the sealant has been widely suggested to improve adhesion to acid etched enamel (4,5,8). Accordingly, the results of the present study revealed that, the application of an intermediate bonding agent layer prior to sealant placement increased the SBS, which was significantly higher in the groups that received a layer of the self-etching adhesive system. A possible explanation for this result would be that the currently available single-bottle adhesives have a great ability to flow deeply into capillary-like spaces of the etched enamel surface and promote an optimal resin tag penetration and enhanced adhesion. The hydrophilic monomers present in the contemporary bonding agents increase the surface wetting and resin penetration (8).

Complete penetration of the etchant into the pits and fissures is essential for sealant retention. However, there have been reports of insufficient penetration of the phosphoric acid etchant into the fissure system (12). Since the use of a hydrophilic adhesive prior to sealant placement improves retention of the sealant and decreases microleakage, the use of self-etching adhesive systems may be a valid and promising alternative to acid etching with phosphoric acid. These adhesives have recently been introduced to simplify the bonding procedures and reduce the technique sensitivity by eliminating the etching, rinsing and drying steps of the bonding protocol (13). The risks of over-etching, over-wetting or over-drying of tooth substrates are thus avoided. However, there is no consensus in the literature regard-

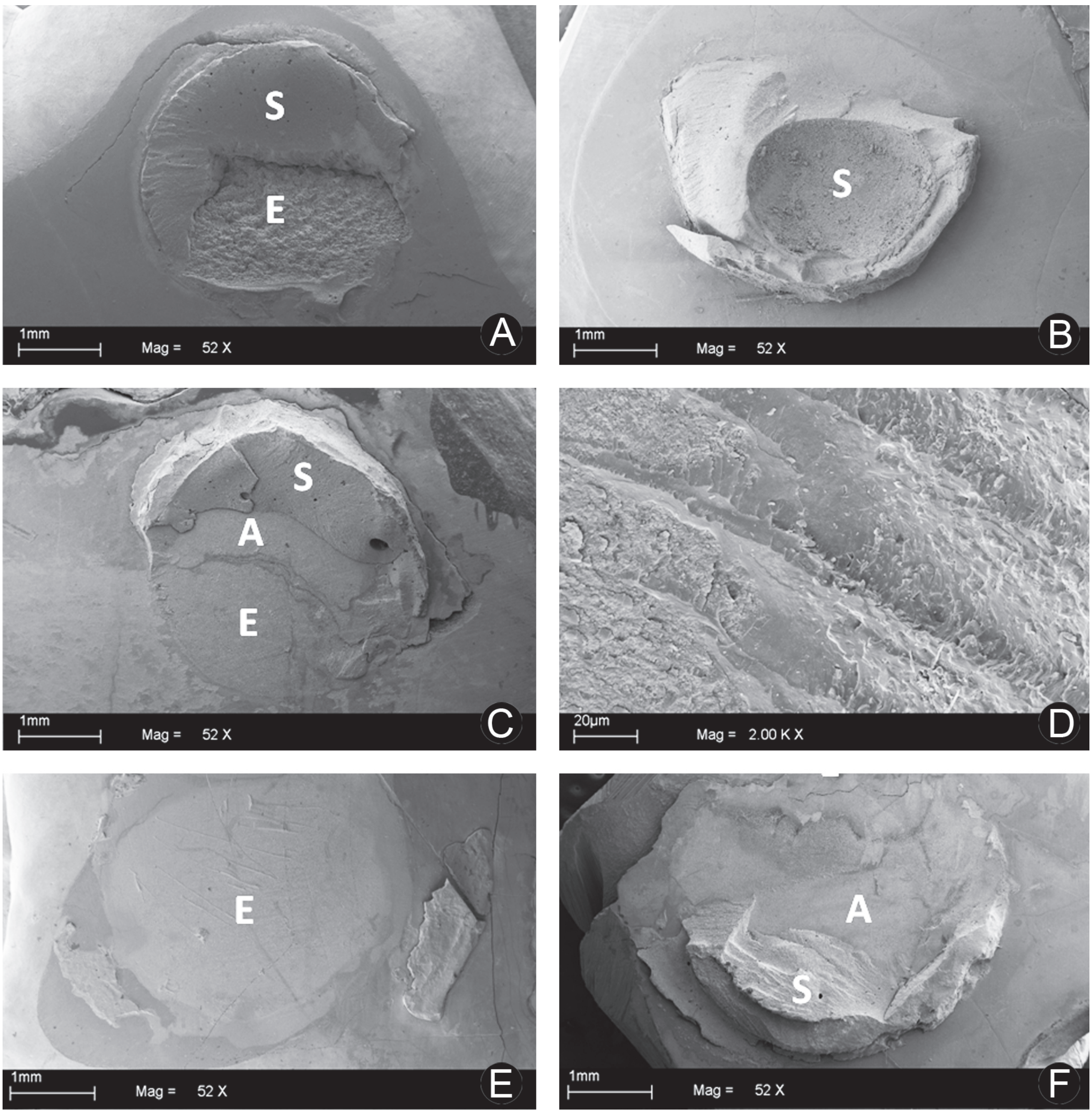

Figure 1. Qualitative analysis of the adhesive interfaces by scanning electron microscopy. A: mixed failure at the sealant/enamel interface (group I); B: cohesive failure in the sealant (group IV); C: mixed failure in which the enamel, adhesive system and sealant can be observed (group II); D: adhesive failure in which the adhesive system can be observed underneath the sealant (group III); E: adhesive failure (group IV); F: mixed failure (group V).

ing the use of mild self-etching adhesives on enamel (15,16). It has been reported that the functional monomers in self-etching adhesives can chemically interact with hydroxyapatite (17), which obviously contributes to the bonding effectiveness to enamel, despite the low etching aggressiveness of the primer. The self-etching adhesive system used in the present study is considered as a weak self-etching primer (pH 2.5) and its hydrophilic acid functional monomer (10-MDP) has an intense chemical interaction with the hydroxyapatite (17). Clinically acceptable retention rates have been reported and may thus be the result of a two-fold mechanism: increased micromechanical retention in addition to the chemical interaction (15). Pashley et al. (18) found no correlation between the bonds to enamel of these adhesive systems and their pH (weak, moderate or strong). So far, the literature does not provide a straight forward answer whether mild self-etch adhesives bonded to enamel can withstand the mechanical and chemical challenges of the oral cavity.

While some clinical and laboratorial studies have shown that the association of self-etching adhesive systems to pit-and-fissure sealants is less effective than the use of etch-and-rinse adhesive systems (14,19), others have reported good results for this association (9,20). The findings of the present study showed that the SBS of the groups in which the Clearfil S3 Bond was applied to enamel surface prior to sealant placement was similar to that of the control group (sealant applied to noncontaminated enamel). In a previous study evaluating the 2-year clinical success when using a self-etching adhesive as the sole etching and adhesive step prior to sealant placement, Feigal and Quelhas (9) concluded that the self-etching adhesive is effective in bonding sealant to enamel and that the simplified protocol shortens dramatically the treatment time and reduces treatment complexity. The authors reported a reduction in clinical chairtime by more than one third when sealants were associated with self-etching adhesive systems, which is a great advantage in pediatric dentistry.

In the present study, the groups in which the sealant was associated with Adper Single Bond presented significantly higher mean SBS compared to the groups in which the sealant was applied alone to contaminated enamel, regardless of the curing protocol. However, the values were significantly lower than those recorded in the control group in which sealant was placed on noncontaminated enamel. Torres et al. (10) reported that the application of a bonding agent (Prime&Bond) layer underneath the resin pit-and-fissure sealant placement resulted in significantly higher SBS to the saliva-contaminated and noncontaminated groups. The authors also found that individual or simultaneous curing of the bonding agent and the sealant had no influence on bond strength to contaminated enamel. The different results found in the present study may be attributed to the fact that Adper Single Bond 2 etch-and-rinse adhesive system contains water as a co-solvent, which gives a lower volatility to this material compared to other adhesive systems that contain only acetone as a solvent. Pedigão et al. (15) evaluated the microtensile bond strength of a self-etching adhesive system associated with Clinpro and Delton pit-and-fissure sealants without salivary contamination and reported that the simultaneous curing of the sealants and the intermediate bonding agent layer produced similar bond strength to that obtained with acid etching. The results of the present investigation showed that the association of the self-etching adhesive system (Clearfil S3 Bond) with the sealant after salivary contamination produced statistically similar mean shear bond strength to that of the control group, regardless of the curing protocol.

Several studies (4,8,10) have documented the advantages of associating adhesive systems with resin sealants. Nevertheless, the literature is scarce in data that support the benefits of associating the contemporary self-etching primer adhesive systems with resin-based sealants as well as the curing protocol (individual or simultaneous), under conditions of salivary contamination. The long-term implications of a single cure on the quality and longevity of the adhesion obtained should also be investigated. From the standpoint of time saving, lowered complexity of treatment, decreased challenge of patient management and uncomfortable delivery, the association of sealants to the new self-etching adhesive systems has a significant advantage over the traditional method and over the association to etch-and-rinse adhesives. The lack of reported studies using the same methodology and materials tested in the present study is a limitation to stating a reliable comparison with outcomes of previous investigations.

It is important to emphasize that this paper in no way suggests that improper technique for sealant placement can be advocated at all. However, even when stringent moisture control procedures are attempted during sealant placement, contamination of acid-etched enamel can occur. Our expectation is that the findings of this study may help improving clinician confidence in sealant success, even in circumstances of application that are far less than ideal.

The following conclusions can be drawn: 1. The curing protocol used for the tested adhesive systems (Clearfil S3 Bond and Adper Single Bond 2) associated to the pit-and-fissure sealant did not affect the SBS to the fresh whole human saliva-contaminated acid-etched enamel; 2. The association of Clearfil S3 Bond self-etching adhesive system to Fluoroshield sealant resulted in significantly higher SBS compared to the use of Adper Single Bond 2 etch-and-rinse adhesive system; 3. The self-etching adhesive system produced similar results to that of the control group, in which the sealant was applied under ideal dry, noncontaminated conditions.

RESUMO

Este estudo avaliou *in vitro* a resistência ao cisalhamento (RC) de um selante resinoso [Fluroshield (F), Dentsply/Caulk] em associação com um sistema adesivo de condicionamento total [Adper Single Bond 2 (SB), 3M/ESPE] ou auto-condicionante [Clearfil S3 Bond (S3), Kuraray Co., Ltd.] após contaminação salivar do esmalte, comparando dois protocolos: fotopolimerização individual do sistema adesivo e do selante ou simultânea de ambos os materiais. Superfícies mesiais e distais de esmalte de 45 terceiros molares hígidos foram aleatoriamente alocadas em 6 grupos (n=15), de acordo com a técnica adesiva empregada: I - F foi aplicado sobre o esmalte condicionado com ácido fosfórico a 37%. Os demais grupos foram contaminados com saliva (0,01 mL por 10 s) após o condicionamento ácido. II - SB e F foram fotopolimerizados separadamente; III - SB e F foram fotopolimerizados simultaneamente; IV - S3 e F foram fotopolimerizados separadamente; V - S3 e F foram fotopolimerizados simultaneamente; VI - F foi aplicado sobre o esmalte condicionado e contaminado sem sistema adesivo. RC foi testada em uma máquina universal de ensaios (0,5 mm/min; 50 kgf) e os dados analisados por ANOVA a 1 fator e teste exato de Fisher (α=0,05). As interfaces adesivas foram analisadas quanto ao padrão de fraturas em estereomicroscópio. Três espécimes de cada grupo foram analisados qualitativamente em microscópio eletrônico de varredura. As médias de RC em MPa foram: I-12,28 (±4,29); II-8,57 (±3,19); III-7,97 (±2,16); IV-12,56 (±3,11); V-11,45 (±3,77); e VI-7,47 (±1,99). Conclui-se que a fotopolimerização individual ou simultânea do sistema adesivo e do selante não afetou os valores de RC ao esmalte contaminado. S3/F apresentou RC estatisticamente maior do que os grupos tratados com o sistema adesivo etch-and-rinse SB e estatisticamente semelhante ao grupo controle, no qual o selante foi aplicado em condições ideais, na ausência de contaminação salivar.

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