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Shear Bond Strength and Microleakage of Pit and Fissure Sealants Placed after Saliva-Contaminated Etched Enamel

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Abstract: Saliva contamination of etched enamel before sealant application is the most common reason for failure of fissure sealants, thus affecting the effect of caries prevention. This study aimed to evaluate the shear bond strength (SBS) and microleakage of resin-based fissure sealant on saliva-contaminated etched enamel after rinsing, re-etching, and applying universal adhesive. Fifty human third molars were sectioned into 2 parts and embedded in acrylic resin to obtained 100 samples. The samples were randomly assigned to 5 groups: 1, etching; 2, etching + contamination; 3, etching + contamination + rinsing; 4, etching + contamination+ re-etching; 5, etching + contamination + universal adhesive. Each group was divided into 2 subgroups: 24 h storage and 5000× thermocycling. After measuring SBS, failure mode was analyzed. In an additional 15 teeth, microleakage was tested using dye penetration method. Three more teeth were used for scanning electron microscope (SEM) observation of the enamel surface morphology in each group. The adhesive group had significantly higher mean SBS after 24 h storage, while the re-etching group were better after 5000× thermocycling. The etching, etching+ contamination+ re-etching, and etching+ contamination+ universal adhesive groups showed the least microleakage. The SEM reveals considerable variations in the enamel surface appearance within groups. Re-etching or applying universal adhesive in saliva-contaminated etched enamel before sealant can achieve satisfactory results. Considering the less operative steps and the shorter chair time, applying universal adhesive is more recommended for a pediatric patient if saliva-contamination happened before sealant application.

Keywords: pit and fissure sealant; saliva contamination; rinsing; re-etching; universal adhesive; shear bond strength; microleakage; scanning electron microscope

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1. Introduction

Dental caries is a disease caused by ecological changes in the composition and activity of the bacterial biofilms when exposed over time to fermentable carbohydrates, resulting in a disturbed balance between demineralization and remineralization [1]. It is the most common chronic disease in children and adolescents. Dental caries also has an influence on adults, with 90% over 20 years old suffering from tooth-root caries to a certain extent [2].

The pits and fissures or anatomical grooves of permanent teeth can catch food debris and promote the formation of bacterial biofilms that increase the risk of dental caries. It has been reported that pit and fissure caries account for 80%–90% of all carious lesions in permanent teeth and 44% in deciduous teeth [3]. Penetrating and sealing the tooth surfaces effectively with dental materials, such as pit and fissure sealants, can prevent dental caries and is part of a comprehensive approach to caries management. Besides prevention of pit and fissure caries, sealants can also inhibit the progression of carious lesions without cavities in order to prevent invasive dental restorations [4,5]. Reported study has shown

that using pit and fissure sealants can reduce the incidence of dental caries by 75% within 2–3 years follow-up compared to those who do not use sealants [6]. Pit and fissure sealing is the most efficient way in preventing dental caries in child and adolescent [7,8].

The sealing material is mechanically bonded to the tooth surface and performs as a physical barrier to bacterial biofilms, thus minimizing the harmful activity of pathogenic microorganisms on the tooth surface. The successful clinical application of fissure sealants depends on their retention, abrasion resistance, and the capacity to maintain adequate sealing on the tooth surface [9,10]. Caries prevention is only possible if the pit and fissure sealant is well adhered and covers all pits and fissures. [11,12]. Cracks in the marginal integrity of the sealing material can subsequently lead to bacterial colonization under the sealant, which can initiate and develop carious lesions [13].

The most important topic during placing sealing material is the ability of a single material to adhere to the solid surface of the tooth [11]. However, dental materials bonding to the tooth substrates were not uncomplicated until Buonocore delivered his classic study, which described a pioneering method of mechanically adhering acrylic resin to dental enamels after phosphoric acid etching [14]. Since then, enamel etching has been considered the gold standard in order to achieve micromechanical bonding of resins and sealants to the tooth surface. The prismatic enamel is exposed after enamel etching, and the resin-based sealants penetrate the microporosities, thus producing resinous tags and mechanical anchoring once polymerized [15,16].

At present, resin-based sealant is the most widely used kind of sealant materials, and has better retention than other materials [11]. According to the manufacturers' instructions, during sealant placement and curing, cleaning and isolation of the enamel surface are usually detailed and a dry environment is encouraged. The enamel surface needs to be acid etched before the resin-based sealant is placed [10]. Keeping the etched enamel surface from being contaminated by saliva is the most crucial step. However, it is usually very difficult to provide an isolated and dry field in small children, patients with special needs, and those with newly erupted teeth [17]. The distal marginal ridge of the teeth is very closed to the retromolar pad, so the occlusal surfaces of the teeth are easily contaminated by saliva in the process of the sealing procedure. It is noted that the main reason for failure of fissure sealant was saliva and moisture contamination of etched enamel during the sealing procedure [9]. This is because the microporosities created by acid etching on the enamel are partially blocked, preventing the formation of optimal resin tag and weakening the bonding of the sealant [10,15,18,19]. Therefore, there is a great need to find a more suitable method to treat the problems of moisture and/or saliva contamination of etched enamel. A study reported that the etched enamel surface morphology after salivary contamination can be re-established through rinsing, re-etching and use of bonding agents before placing the sealant [20]. However, the effects of rinsing, re-etching, and applying universal adhesive on the saliva-contaminated etched enamel are still not fully clear.

Sealant retention is usually evaluated by the shear bond strength (SBS) test and the results are considered to be reliable and valid in the comparative study of determining the adhesion between the dental material and tooth structure [21]. Microleakage tests are designed to evaluate the sealing ability by marking the permeable area around the restoration or sealant material with a penetrant [13,22]. Methylene blue, fuchsine, and silver nitrate were the most commonly used penetrants, with no significant differences. The advantages of fuchsine are that it is non-toxic and inexpensive [23].

The aim of this study is to evaluate the shear bond strength (SBS) and microleakage of a resin-based sealant to non-contaminated and saliva-contaminated etched enamel treated by rinsing, re-etching, and applying a universal adhesive, thus providing a reference for the selection of more appropriate countermeasures for saliva-contaminated etched enamel during pit and fissure sealing. The null hypothesis of this research was that there are no differences between the SBS and microleakage of resin-based fissure sealant on saliva-contaminated etched enamel after rinsing, re-etching, and applying universal adhesive.

2. Materials and Methods

2.1. Sample Preparation

This study was approved by the ethical committee of School of Stomatology, China Medical University (protocol code: 202123, date of approval: 4 June 2021). Written informed consents were also obtained from the study participants.

A diagram of the experimental design is shown in Figure 1. Fifty healthy, caries-free, extracted human third molars were used in the investigation for testing SBS. All of the teeth were without any developmental abnormalities, visible fractures or cracks, fillings, fissure sealants, and had finished root development. Sclerotic teeth with a dark appearance and covered with dental calculus from seniors were excluded. After extraction, the teeth were thoroughly washed in running water and all blood and adherent tissue were removed. After being soaked in 1.0% chloramine-T trihydrate solution in room temperature for 24 h, the teeth were stored in normal saline solution at 4 °C. The solution was replaced at least once a month to minimize deterioration. All the teeth used in this research were stored for no more than 6 months. The roots were excised 1 mm apically from the cementoenamel junction, and the crowns were longitudinally divided into two parts (buccal, lingual) with a diamond disc (Dental Diamond Disc, Shofu INC, Kyoto, Japan) [24].

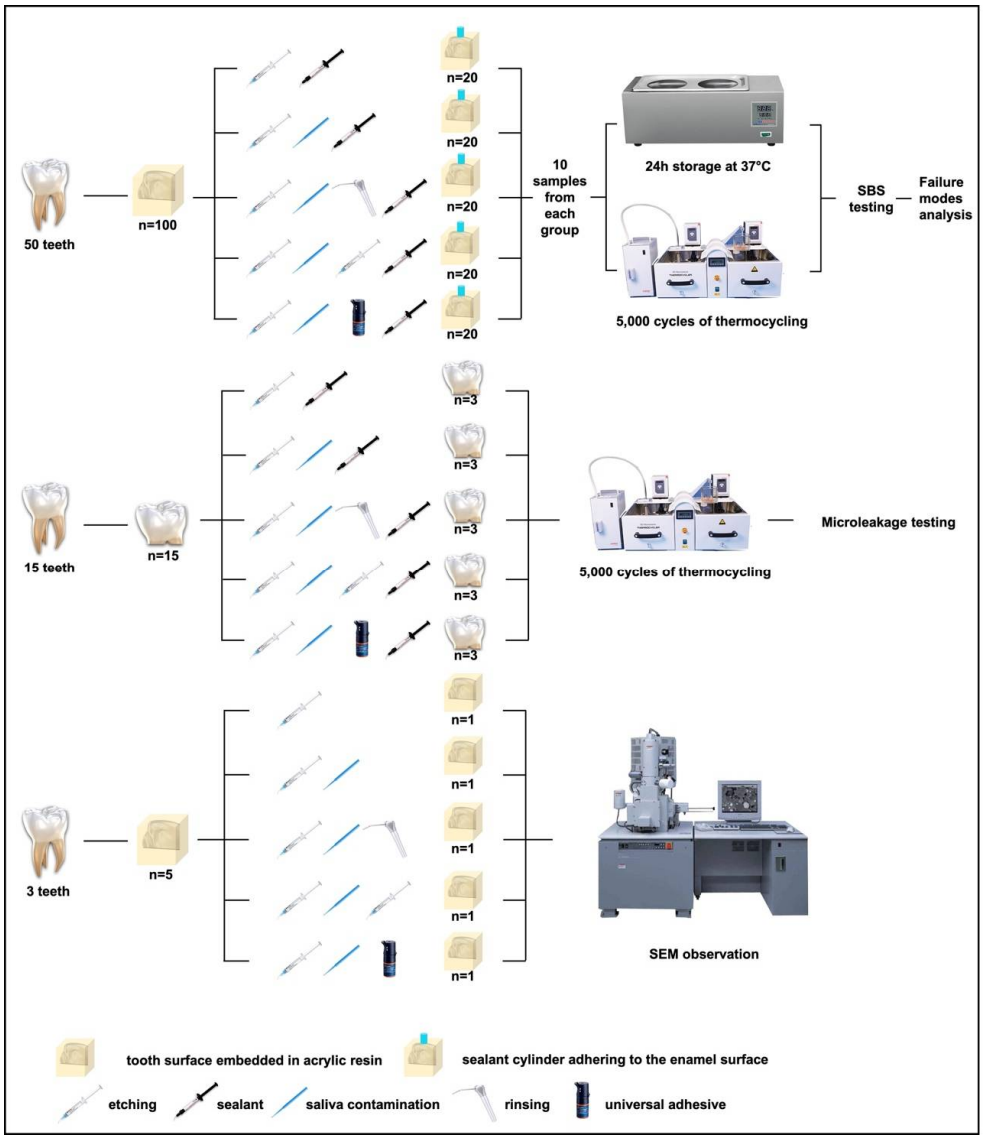

Figure 1. Diagram of the experimental design. SBS, Shear bond strength.

The prepared tooth surfaces were embedded in a self-curing acrylic resin by cube silicone mold with a side length of 1.5 cm (Figure 2a). In order to make sure the enamel surface of the samples was parallel to the top surface, the enamel surface of each sample was pre-grounded with 120-grade silicon carbide sandpaper to ensure the planeness, and was adhered to the bottom of the mold with dental wax to prevent movement during the embedding process. The embedded samples were ground with a water-cooled mechanical polisher and 120- and 400-grit silicon carbide abrasive paper to expose a flat area of about 3 mm in diameter on the enamel surface [24]. This process resulted in 100 specimens, which were randomly assigned to 10 study groups (n = 10) (Figure 1). We numbered the samples on the basis of the randomization table. The procedures for each group are described in Table 1.

Table 1. Distribution of the groups.

Group	Contamination	Rinsing/Re-Etching/ Universal Adhesive	24 h Storage at 37 °C/5000 Cycles of Thermocycling
1	No	-	24 h storage at 37 °C
1′	No	-	5000 cycles of thermocycling
2	Yes	-	24 h storage at 37 °C
2′	Yes	-	5000 cycles of thermocycling
3	Yes	Rinsing	24 h storage at 37 °C
3′	Yes	Rinsing	5000 cycles of thermocycling
4	Yes	Re-etching	24 h storage at 37 °C
4′	Yes	Re-etching	5000 cycles of thermocycling
5	Yes	Universal Adhesive	24 h storage at 37 °C
5′	Yes	Universal Adhesive	5000 cycles of thermocycling

2.2. Placing the Fissure Sealant Material

The use of pit and fissure sealants in vitro was in strict accordance with the manufacturer's clinical recommendations. The enamel surfaces were first cleaned with a prophylaxis paste without fluoride (Pressage, Shofu Inc., Kyoto, Japan) and washed with copious water and dried with water- and oil-free air.

For all the samples, an etching procedure with 37% phosphoric acid gel (Scotchbond™ Universal Etchant, Etching Gel, 3M Deutschland, Neuss, Germany) was performed for 30 s. The tooth surface was then rinsed with a water spray for 30 s and dried with pressurized air for 10 s until it showed a characteristic frosty appearance. We performed the bonding procedure in each group as follows: A polypropylene mold with a central hole 2 mm in diameter × 3 mm height was applied over the enamel surface and fixed with wax (Figure 2b). The fissure sealant (Clinpro™ Sealant, Polymer-based pit and fissure sealants, 3M ESPE, St. Paul, MN, USA) was inserted into the mold, using a syringe with a dispensing syringe tip to avoid the inclusion of air bubbles and followed by photopolymerization for 20 s each with a light-curing unit (Miniled™, Setelec S.A.S, ACTEON Group, Merignac Cedex, France). The light intensity in the unit was measured periodically and ranged from 900 to 1200 mW/cm^2, which meets the requirement of the sealant materials. Then, the mold was removed carefully, leaving a sealant cylinder (2 mm × 3 mm) adhering to the enamel surface (Figure 2c). After bonding procedure, the samples were stored in distilled water at 37 ± 1 °C in a thermal oven for 24 h. In groups 1′, 2′, 3′, 4′, and 5′, after 24 h storage in distilled water at 37 °C, the samples were followed by thermocycling (Thermocycler, the 1100, SD Mechatronik GmbH, Feidkirchen-Westerham, Germany) between 5 °C (±2 °C) and 55 °C (±2 °C) for 5000 cycles [25], with a dwell time of 30 s and a transfer time of 5 s. Next, they were subjected to the SBS test.

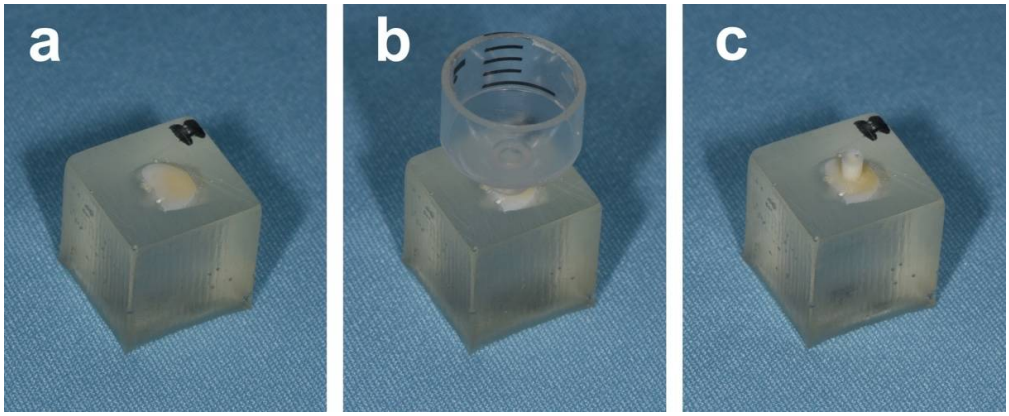

Figure 2. The process of sealant cylinder adhering to the enamel surface. (**a**) The tooth surface was embedded in self-curing acrylic resin; (**b**) a polypropylene mold with a central hole 2 mm in diameter × 3 mm height was applied over the enamel surface and fixed with wax; (**c**) the mold was removed, leaving a sealant cylinder adhering to the enamel surface.

The groups with saliva contamination were contaminated by the operator's integral and fresh saliva. The unstimulated saliva was collected 1 h after food or drink consumption. After enamel etching and drying, 0.01 mL of the saliva was applied onto the whole surface area of the enamel with a micropipette, and was left undisturbed for 30 s.

In groups 2 and 2′, after saliva contamination, the samples were completely dried with air spray. Then, sealant cylinder adhesion to the enamel surface was performed, as mentioned above.

In groups 3 and 3′, after saliva contamination, the samples were rinsed with a water spray for 30 s and completely dried with air spray. Then, sealant cylinder adhesion to the enamel surface was performed, as mentioned above.

In groups 4 and 4′, after saliva contamination, the samples were completely dried with air spray. Then, 30 s of re-etching and sealant cylinder adhesion to the enamel surface was performed, as mentioned above.

In groups 5 and 5′, after saliva contamination, the samples were completely dried with air spray. Single Bond Universal Adhesive (3M/ESPE, St. Paul, MN, USA) was applied to the enamel surface with disposable microbrush tips and rubbed in for 20 s, the adhesive was gently air dried for approximately 5 s, and then the sealant cylinder adhesion to the enamel surface was performed, as mentioned above.

2.3. Notched-Edge SBS Testing and Failure Mode Analysis

The notched-edge SBS test was carried out using a computer-controlled servo-hydraulic testing machine (CARE M-3000, Kell measurement and control Co., Ltd., Tianjin, China). First, the samples were held in a metal sample holder. A notched-edge shear fixture with a semicircular molded shear blade was mounted on the testing machine and placed over the sealant cylinder on the aligned specimen. The notched-edge shear blade had to be positioned exactly over the cylinder and force fitted without premature contact to ensure that the load was applied directly to the cylinder. A constant crosshead speed of 0.5 mm/min was applied until the material failed. We recorded the maximum load force (N) of each specimen at failure. The calculation method of SBS is to divide the maximum load force by the bonded area, that is, SBS (MPa) = load (N) / area (mm^2), where the bonding area in this study is π mm^2.

The failure modes of all the samples were examined using a dental operating microscope (Suzhou Semorr Medical Technology Co., Ltd., Suzhou, China) with 20-fold magnification. The failure mode was described as follows: 1. adhesive, at the enamel interface/sealant or adhesive system; 2. cohesive, in the body of the sealant or enamel, maintaining an intact interface; 3. mixed, when disruption of the adhesive bond and the material or the substrate occurred at the same time.

2.4. Microleakage Testing

Fifteen human third molars were used in microleakage testing; that is, three teeth in each of the five groups. We stored and cleaned the teeth as previously mentioned. Fissure sealing of the whole teeth was performed in strict accordance with the manufacturer's clinical recommendations after treating the enamel surface differently in each group. Every sample was placed in distilled water at 37 °C for 24 h followed by a thermocycling bath, as mentioned above. When thermocycling ended, the root surfaces were covered with dental wax (Base plate Wax, Kunshan, China). Then, we brushed two layers of nail varnish onto the whole tooth surface, except for the area within 1 mm of the fissure sealant. The varnish was used to prevent the dye from penetrating other parts of the tooth. The samples were immersed in 0.5% fuchsin basic-ethanol solution (Phygene biotechnology Co., Ltd., Fuzhou, China) for 24 h at 37 °C and then thoroughly rinsed in tap water [24].

The roots were cut off 1 mm below the cementoenamel junction using a diamond disc. To prevent the sealant from cracking, the tooth crowns were then fully embedded in self curing acrylic resin. We cut the prepared resin block into at least five pieces along the buccal-lingual direction of the crown, and the thickness of each piece was about 1 mm. The front and back sides of each slice were used for inspection, and 10 sides were obtained for each tooth. The slices were observed using a dental operating microscope with a 20-fold magnification by an examiner blinded to the treatment of the tooth. Each observation surface was photographed using a digital camera [24]. The penetration depth of the dye into the fissure sealants on each picture was analyzed, and pictures without pit and fissure sealants were excluded.

The criterion for the amount of dye microleakage was the maximum level of dye penetration. Scoring was based on the following criteria [20]:

Grade 0: No penetration;

Grade 1: Dye penetration up to one-third of the sealant-tooth interface;

Grade 2: Dye penetration extending from one-third to two-thirds of the length of the sealant- tooth interface;

Grade 3: Dye penetration more than two-thirds of the length of the sealant-tooth interface.

2.5. SEM Observation

Three human third molars were treated as mentioned in the sample preparation to get six specimens, and five of the specimens were used in this part. Different treatment of the enamel surface was performed according to the distribution of the groups.

Immediately after conditioning, the specimens were dried at room temperature, sprayed with gold coating, and fixed to aluminum stubs with adhesive carbon tape. The analysis was performed using a scanning electron microscope (Zeiss Merlin Compact, Zeiss, Jena, Germany) in low vacuum at 10 Pa of chamber pressure, with an electron acceleration voltage of 20 kV and detecting backscattered electrons. Enamel surface morphology and etching patterns were observed at ×500, ×1000, and ×2000 magnification.

2.6. Statistical Analysis

SPSS 22.0 software was used for statistical analysis of the data. Normally distributed measurement data are expressed as "mean ± standard deviation". Comparisons of the SBS values of the 5 groups with 24 h storage at 37 °C or 5000 cycles of thermocycling are performed by the Least Significant Difference (LSD) test. The paired t-test was used for comparison of the SBS values in the groups with 24 h storage at 37 °C and after 5000 cycles of thermocycling. $p < 0.05$ indicates that the difference is statistically significant. Data analysis of the microleak testing's results was performed using the chi-square test and Fisher's exact probability results. $p < 0.001$ indicates that the difference is statistically significant.

3. Results

3.1. Notched-Edge SBS Testing

The shear bond strength values of each specimen, mean values, and standard deviation (S.D) of each group and LSD test results are listed in Table 2.

Table 2. Mean values, standard deviation (SD), and Least Significant Difference test results for shear bond strength values (MPa) in each group.

Group	Mean ± SD	Comparison of the Group	*p*-Value
1	17.40 ± 6.36	1 vs. 2	0.815
		1 vs. 3	0.369
		1 vs. 4	0.028 *
		1 vs. 5	0.000 *
2	16.93 ± 4.14	2 vs. 1	0.815
		2 vs. 3	0.259
		2 vs. 4	0.016 *
		2 vs. 5	0.000 *
3	19.22 ± 3.19	3 vs. 1	0.369
		3 vs. 2	0.259
		3 vs. 4	0.181
		3 vs. 5	0.000 *
4	21.96 ± 4.94	4 vs. 1	0.028 *
		4 vs. 2	0.016 *
		4 vs. 3	0.181
		4 vs. 5	0.002 *
5	28.61 ± 2.96	5 vs. 1	0.000 *
		5 vs. 2	0.000 *
		5 vs. 3	0.000 *
		5 vs. 4	0.002 *
1′	12.79 ± 2.13	1′ vs. 2′	0.000 *
		1′ vs. 3′	0.097
		1′ vs. 4′	0.018 *
		1′ vs. 5′	0.099
2′	8.55 ± 1.84	2′ vs. 1′	0.000 *
		2′ vs. 3′	0.044 *
		2′ vs. 4′	0.000 *
		2′ vs. 5′	0.000 *
3′	10.88 ± 2.31	3′ vs. 1′	0.097
		3′ vs. 2′	0.044 *
		3′ vs. 4′	0.000 *
		3′ vs. 5′	0.001 *
4′	15.56 ± 3.73	4′ vs. 1′	0.018 *
		4′ vs. 2′	0.000*
		4′ vs. 3′	0.000 *
		4′ vs. 5′	0.444
5′	14.69 ± 2.13	5′ vs. 1′	0.099
		5′ vs. 2′	0.000 *
		5′ vs. 3′	0.001 *
		5′ vs. 4′	0.444

* Significant *p*-value, indicate statistically significant differences in the SBS between the groups (*p*-value < 0.05).

After 24 h storage at 37 °C, the highest SBS values were obtained for group 5, when the sealants were combined with universal adhesive in saliva-contaminated enamel. The SBS were significantly higher than that in other groups. Although the average value of bond strength in group 3 was higher than that in group 2, the difference was not statistically significant, which means rinsing did not eliminate the harmful effects of saliva contamination on etched enamel.

After 5000 cycles of thermocycling, the situation seems different. The results of the t-test showed that the SBS value of each group was significantly reduced, and the difference was

statistically significant. Re-etching showed the best performance on saliva-contaminated enamel; the bond strength was even higher than that in the group without contamination, and the difference was statistically significant. In the case of saliva contamination, application of the adhesives eliminated the adverse effects of saliva contamination, and the SBS values were not statistically different from the uncontaminated group. There was also no statistically significant difference between the groups of re-etching and universal adhesive.

3.2. Failure Mode Analysis

The fracture patterns and distribution of failure modes in each group are shown in Figures 3–5. After 24 h of storage at 37 °C, the most common type of failure in the saliva-contaminated group was adhesive failure. The mixed failure was the dominant type in other groups. The cohesive failure was the least in all of the groups, and there was one specimen in the standard group and the re-etching group respectively.

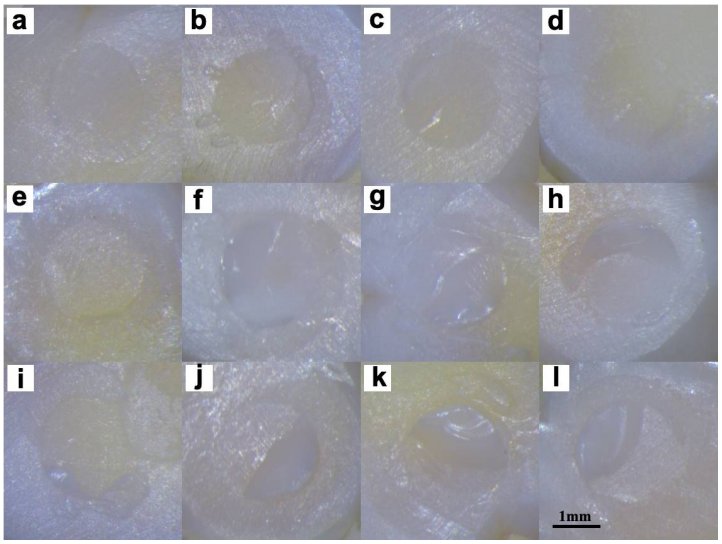

Figure 3. Failure modes of the samples after 24 h storage at 37 °C by operation microscope with 20-fold magnification. (**a**–**e**) Adhesive failure in groups 1, 2, 3, 4, and 5; (**f**,**g**) cohesive failure in the sealant in groups 1 and 4; (**h**–**l**) mixed failure in groups 1, 2, 3, 4, and 5.

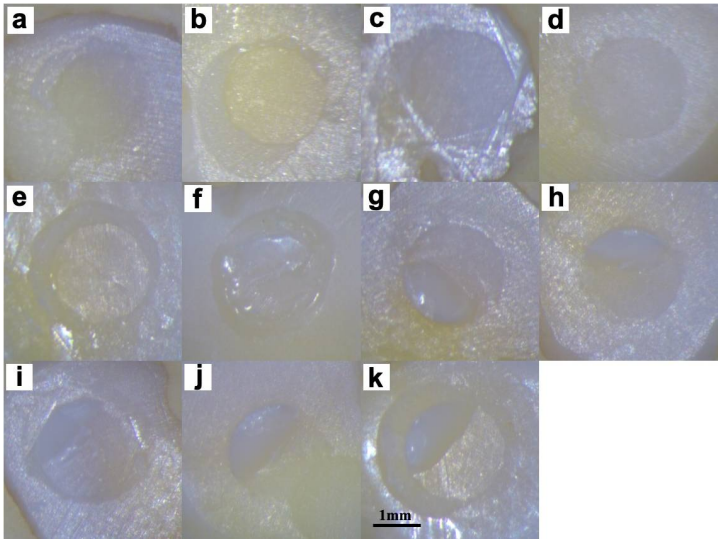

Figure 4. Failure modes of the samples after 5000 cycles of thermocycling by operation microscope with 20-fold magnification. (**a**–**e**) Adhesive failure in groups 1′, 2′, 3′, 4′, and 5′; (**f**) cohesive failure in the sealant in group 4′; (**g**–**k**) mixed failure in groups 1′, 2′, 3′, 4′, and 5′.

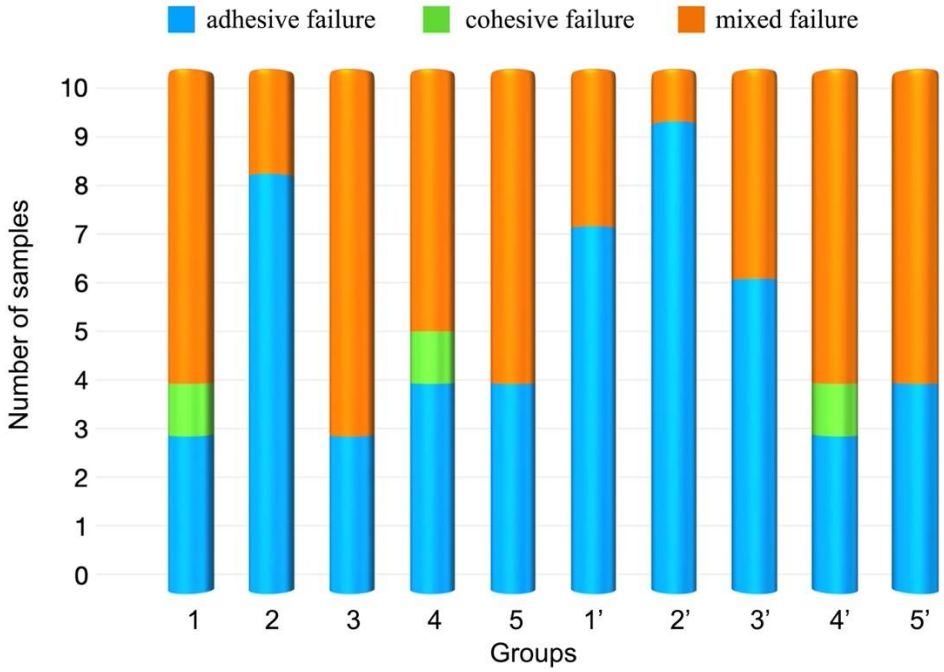

Figure 5. Distribution of failure modes in each group. Group 1, etching; group 2, etching + contamination; group 3, etching + contamination + rinsing; group 4, etching + contamination+ re-etching; group 5, etching + contamination + universal adhesive. For groups 1–5, 24 h storage; groups 1′–5′, 5000× thermocycling.

After 5000 cycles of thermocycling, adhesive failure was the most common type of failure in the standard group, saliva-contaminated group, and rinsing group, whereas mixed failure was the dominant in the re-etching and adhesive group. Only one cohesive failure was observed in the re-etching group.

3.3. Microleakage Testing

Varying degree microleakage between the enamel surface and the fissure sealant were shown in Figure 6. A total of 143 tooth slides with fissure sealants were examined for microleakage. The percentage of each score for all groups has been shown in Table 3. The results of the pairwise comparison are shown in Table 4. There is a statistical significance between groups 1′, 4′, and 5′ with group 2′, no difference between groups 3′ and 2′, and no difference between groups 1′, 3′, 4′, and 5′.

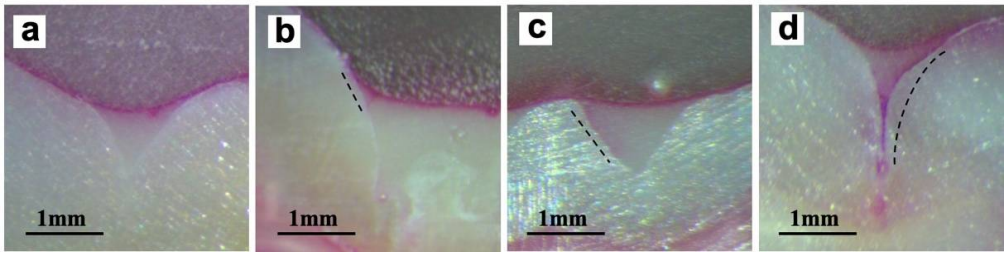

Figure 6. Microscopic images showing microleakage between the enamel surface and the fissure sealant (20×). (**a**) No dye penetration; (**b**) dye penetration up to one-half or less of the sealant depth; (**c**) dye penetration extending to the inner half of the sealant; (**d**) dye penetration to the sealant base. The dotted lines indicate dye penetration.

Table 3. Microleakage of the fissure sealants following 5000 cycles of thermocycling.

Group	Number of Teeth (N)	Number of all Available Tooth Slides with Fissure Sealants N (%)	Grade 0	Grade 1	Grade 2	Grade 3
1′	3	30 (100.00)	28 (93.33)	0	1 (3.33)	1 (3.33)
2′	3	29 (100.00)	17 (58.62)	1 (3.45)	0	11 (37.93)
3′	3	29 (100.00)	26 (89.66)	0	0	3 (10.34
4′	3	27 (100.00)	25 (92.59)	1 (3.70)	0	1 (3.70)
5′	3	28 (100.00)	27 (96.43)	0	0	1 (3.57)

Table 4. Pairwise comparison of the five groups of the microleak testing results.

			Group				
			1′	2′	3′	4′	5′
Grade	0	Number	28 [a]	17 [b]	26 [a,b]	25 [a]	27 [a]
		% within group	93.30%	58.60%	89.70%	92.60%	96.40%
	1–3	Number	2 [a]	12 [b]	3 [a,b]	2 [a]	1 [a]
		% within group	6.70%	41.40%	10.30%	7.40%	3.60%

Superscript [a,b] represents the statistically significant differences between groups in the microleakage test (Chi-square test, Fisher's exact probability result, $p < 0.001$).

3.4. SEM Observation

The electron microscope reveals considerable variations in enamel surface appearance within groups. Etched enamel showed a characteristic honeycomb etching pattern, with generalized surface roughening (Figure 7a–c). After being contaminated by saliva, the surface topography was altered dramatically, and is covered by an organic adhesion layer (Figure 7d–f). Although rinsing removes a small amount of saliva contamination residue, the surface still shows the presence of an adherent layer that covers the etched region (Figure 7g–i). Re-etching significantly altered the saliva-contaminated enamel surface, re-establishing the etched enamel surface morphology. Non-residue of saliva contamination was observed after re-etching (Figure 7j–l). After applying the universal adhesive, the enamel surface was covered with a bonding agent (Figure 7m–o).

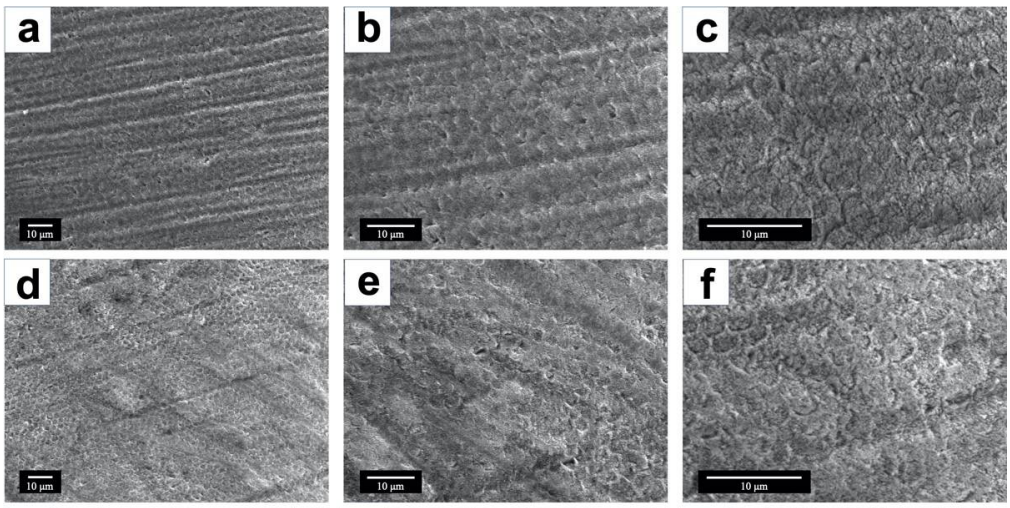

Figure 7. *Cont.*

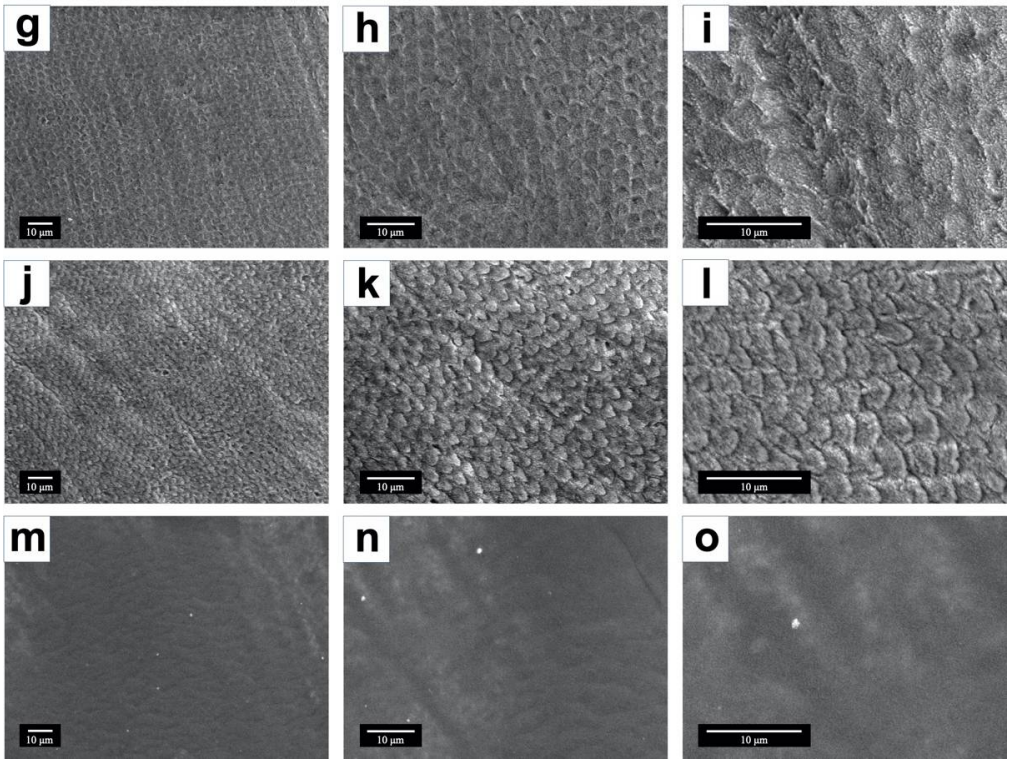

Figure 7. Scanning electron microscope images of the enamel surface after treatment in each group. (**a**–**c**) Etched enamel in groups 1 and 1′ (magnification ×500, 1000, 2000); (**d**–**f**) etched enamel that has been saliva-contaminated in groups 2 and 2′ (magnification ×500, 1000, 2000); (**g**–**i**) etched enamel that has been saliva-contaminated and rinsed in groups 3 and 3′ (magnification ×500, 1000, 2000); (**j**–**l**) etched enamel that has been saliva-contaminated and re-etched in groups 4 and 4′ (magnification ×500, 1000, 2000); (**m**–**o**) etched enamel that has been saliva-contaminated and applied with adhesive in groups 5 and 5′ (magnification ×500, 1000, 2000).

4. Discussion

According to the reported studies, the SBS value of the fissure sealant applied to non-contaminated etched enamel varies from 7.72 ± 0.41 to 14.60 ± 0.30 [9,16,26,27], which is 17.40 ± 6.36 after 24 h storage and 12.79 ± 2.13 after thermocycling in our search. This variation may be due to different pit and fissure sealant materials used and different methods of the accelerated ageing test. In the condition of saliva contamination, the SBS value decreased significantly, which is consistent with reported results [9,16,26]. The SBS values of the saliva-contaminated samples after vigorous rinsing and uncontaminated samples were not statistically different in short term, which is consistent with previous results [27], but were statistically different after thermocycling in our research. To our study and reported results, the sealant in samples with 30 s saliva contaminated could achieve even higher SBS values than that applied to etched uncontaminated dry enamels after 15–30 s re-etching [27]. After using the self-etching adhesive, saliva-contaminated groups can achieve similar SBS values to those of the uncontaminated samples in control groups, which is consistent with previous studies [16,26,28]. Based on analysis of the fracture mode, groups with more mixed failures and cohesive failures showed better bonding than those with more adhesive failures. This result is consistent with that of the SBS test and similar with the studies that have been reported [9,16,26,27]. According to our findings and previous reports, saliva contamination significantly increases the microleakage of pit and fissure sealant [20,26,29]. The effect of saliva contamination on microleakage can be eliminated after using nano-filled bonding agents or universal adhesive before applying the sealant [20,29]. Our results show that re-etching also eliminates the adverse effect of saliva contamination on microleakage, and rinsing may improve results, but not statistically.

During the clinical procedure of pit and fissure sealing, it is crucial to avoid saliva contamination of the etched enamel surface before placing the sealant materials [7]. Using a rubber dam may be the most reliable technique for tooth isolation during clinical procedure [5,7] but may not be feasible when tooth is not fully erupted or when the patient is not cooperative [30]. Cotton rolls, high-volume evacuation with compressed air, or absorbent shields are also a choice [31]. Unfortunately, contamination cannot always be controlled. As newly erupted teeth are less mineralized and therefore more susceptible to acid attack, the optimal time for the application of fissure sealants is immediately after tooth eruption [16]. Paradoxically, the distal marginal ridge is so close to the retromolar pad or gingival margin shortly after tooth eruption, which leaves the tooth surface at high risk of moisture and saliva contamination during the sealing procedure [32]. Moreover, the patient's cooperation may be weak at a younger age, considering the multiple steps involved in the placement of sealants, where contamination during tongue movements and swallowing can still occur [33]. Once saliva contamination happens, the organic adherent pellicle formed on the etched enamel will partially occlude the enamel pores, which impairs the penetration of the sealant into the microporosities created by etching, thus averting formation of the resin tags in charge of mechanical retention [15,18]. Inadequate isolation leads to unnoticed salivary contamination or the presence of moisture in the operative field frequently, then the bond strength between the sealant material and the contaminated surface, which might lead to partial or total loss of the sealant restoration within a short time [34].

Because the organic ingredients in saliva penetrated into microporosities are created by etching, neither cotton pellet nor air-drying effectively eliminate the side effects of saliva from the contaminated enamel surface [26]. Meanwhile, it has been demonstrated that rinsing of the contaminated etched surface with water is not enough to remove the organic debris and protein left by saliva [9,15,18]. However, some researchers conclude that the bond strength between sealant and saliva-contaminated specimens after vigorous rinsing is not found to be significantly different from the bond strength to non-contaminated enamel [19,27]. If the sealant to enamel bond is intact on some surfaces of the contaminated etched enamel, but not all, the bond strength can be maintained in vitro for a short period of time [34]. However, the long-term effectiveness of sealing ability may not be guaranteed. Thermocycling between 5 °C and 55 °C is one of the commonly used methods as an accelerated ageing test, which is carried out to simulate the thermal changes taking place in oral environment [13,25]. According to the findings of this research, vigorous rinsing of the saliva-contaminated etched enamel may improve the effect of the sealant for a long period but it is not good enough.

In order to overcome the contamination problem by saliva, re-etching of the contaminated enamel is recommended by some authors to ensure adequate bonding of the resin material [15,19,35]. Without additional mechanical preparation, re-etching provides the expected bond strength sufficiently [35]. Increasing the re-etching time for 15 s or more will reduce the potential risk of sealant failure [27]. There is a trend towards higher SBS values of sealants to contaminated enamel with increasing re-etch time, although the difference is not significant [27]. However, the shorter the etching time, the less chance of saliva contamination, especially in children who do not cooperate well [7]. Re-etching of the contaminated enamel could also increase the microtensile bond strength significantly and is the optimal method for eliminating the negative impacts of saliva contamination [36]. It is worth mentioning that acid etching with phosphoric acid does not necessarily produce a uniform and fully etched surface; many factors such as the kind of acid agent, acid concentration, etching duration, and enamel surface composition affect the quantity and quality of etching [37]. In this study, it has been shown that the standard deviation of the SBS value is relatively large in the groups treated by etching or re-etching, which indicates the inhomogeneity of this treatment method. No matter the short period or after thermocycling, the re-etching group has achieved satisfactory results.

Feigal et al. first brought up in 1993 the idea of using adhesives under sealants when they used hydrophilic adhesives to enhance the bonding strength when applying

sealants in humid environments [34]. Some research has revealed the positive impact of adhesive systems on the retention of the fissure sealant. The adhesive's component may increase the penetration into enamel porosities, so as to improve the adhesive strength. Self-etch adhesive systems are inferior to etch-and-rinse adhesive systems in terms of sealant retention [5,38,39]. A study has shown that the use of adhesives when performing pit and fissure sealing did not significantly improve sealant retention and reduce marginal discoloration. Considering the complexity and cost of the clinical operation, routine use of adhesives during the sealing process is not necessary [40].

However, under the condition of salivary contamination, the application of adhesives on the etched enamel surface increases the bond strength, decreases the microleakage, and enhances the resin flow into the fissures, which benefits the clinical success of fissure sealant therapy [16,41–43]. Acetone-based solvents and hydrophilic monomers can replace the saliva and penetrate the porosities in these systems, thus improving the combination of sealant materials and saliva-contaminated enamel [41]. In our research, application of adhesive on the contaminated etched enamel achieved similar results with those after re-etching, whether in a short period or after thermocycling.

One of the advantages of using adhesive is the elimination of rinsing steps, which reduces the clinical work time and helps to provide an isolated environment [44]. Moreover, in the face of the current pandemic of COVID-19, elimination of the rinsing step will reduce the risk of cross infections and the time of potential exposure to pathogens [45]. A study showed that curing of the etch-and-rinse adhesive system and sealant separately or simultaneously had no effect on the bond strength to enamel after salivary contamination [42]. After applying adhesive and sealant material, simultaneous curing will further save on clinical working time. A shorter chair time and fewer operative steps are particularly attractive when treating pediatric patients [16]. The universal adhesives or the multi-mode adhesives are the most popular type in adhesive dentistry. This kind of adhesive system can be used as an etch and rinse adhesive, a self-etch adhesive, or to do self-etch on dentin and etch-and-rinse on enamel, which is called selective enamel etching. The composition of these kind of adhesive systems are different from the other adhesive systems, which allow chemical and micromechanical bonding [46]. For the dental community, these new universal adhesive systems are user-friendly, the bonding procedures are simplified, and the adhesive technique sensitivity is reduced.

The limitation of the study is that, in a clinical situation, the fissure sealants are applied on newly erupted tooth surfaces with prismless enamel, while in vitro studies typically use ground flat tooth surfaces, whose prismless enamel are mechanically removed. This may not fully simulate the clinical situation. Prospective or retrospective clinical studies are needed to validate the findings of this experiment. With the progress of scientific research, non-invasive examination such as ultrasound may be used to examine the clinical effect of pit and fissure sealants in clinical practice [47].

5. Conclusions

Whether it is re-etching or applying a universal adhesive in saliva-contaminated etched enamel before the sealant can achieve satisfactory results. Considering the fewer operative steps and shorter chair time, applying a universal adhesive is more recommended in pediatric patient if saliva-contamination were to occur.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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