Original Article

Effect of Saliva Contamination on Microleakage Beneath Bonded Brackets: A Comparison Between Two Moisture-Tolerant Bonding Systems

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Abstract

Objectives: This study aimed to evaluate the effects of saliva contamination on the metallic bracket microleakage bonded with two moisture-tolerant bonding systems.

Materials and Methods: Ninety freshly extracted premolar teeth were randomly divided into six groups of 15 with the following treatments: G1 (control): After acid etching, Assure primer and Assure adhesive were applied to non-contaminated enamel surfaces. G2 (contaminated after etching): The etched enamel surface was exposed to saliva, then Assure primer and Assure adhesive were applied. G3 (contaminated after priming): Saliva contamination was done after application of Assure primer. The exact same procedures were applied to groups G4 to G6 except that TIMP primer and Transbond Plus adhesive system were used.

To measure the microleakage score, the teeth were stained with 2% methylene blue for 24 hours, sectioned and examined under a stereomicroscope at ×16 magnification. Data analysis was performed using Fisher's exact test.

Results: In dry conditions, Assure and TMIP were not significantly different in terms of microleakage scores. All contaminated groups exhibited higher microleakage score at the enamel/adhesive interface compared to the bracket/adhesive interface ($P < 0.01$). In wet conditions, Assure groups showed higher microleakage at the enamel-adhesive interface compared to the TMIP groups ($P<0.05$). At the bracket-adhesive interface, the microleakage scores were not significantly different in saliva contaminated groups compared to the controls.

Conclusion: Saliva contamination caused greater microleakage at the enamel-adhesive interface compared to the adhesive-bracket interface.

Keywords: Orthodontic Brackets; Adhesives; Saliva

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INTRODUCTION

In modern orthodontics, resin-based composites are widely used for bracket bonding. One disadvantage of these resins is polymerization shrinkage, which can lead to leakage of bacteria and fluid through the tooth

–adhesive and bracket-adhesive interfaces [1,2]. Microleakage beneath the orthodontic brackets may be followed by enamel discoloration and decalcification as well as decreased bond strength. Enamel decalcification (white spot lesion) is one of the main concerns of patients receiving fixed orthodontic therapy [3]. This lesion can develop within four weeks in patients with poor oral hygiene [4].

On the other hand, one of the common problems during bracket bonding is poor isolation [5,6], which can result in surface contamination by saliva, blood, gingival crevicular fluid, or water [7]. Saliva contact with the etched tooth results in plugging of porosities produced by acid etching; therefore the micromechanical retention and bond strength of adhesive may decrease [8,9]. To overcome the clinical problems related to poor isolation, some manufacturers introduced hydrophilic bonding materials, which can be used on moistened surfaces. Transbond Moisture Intensive Primer (TMIP; 3M Unitek) + Transbond Plus Color Change Adhesive (3M Unitek) and Assure Primer (Reliance Orthodontic Products) + Assure Adhesive Paste (Reliance Orthodontic Products) are two examples, which have been reported to have higher bond strength on saliva or water contaminated surfaces compared with conventional or hydrophobic systems [10,11].

Many studies have evaluated the effect of saliva contamination on bond strength of hydrophilic adhesive systems; however, we could not find any study assessing the effect of saliva contamination during bonding process on microleakage beneath bonded brackets. Hence, we aimed to study the effect of saliva contamination on microleakage score of brackets bonded with two hydrophilic adhesive systems.

MATERIALS AND METHODS

Ninety intact premolars, extracted for orthodontic purposes, were used in this study. The teeth were cleaned thoroughly under running water before storage. Then, scaling and rubber cup prophylaxis with pumice paste were carried out and the teeth were rinsed with water and air-dried. The samples were randomly divided into six groups of 15. The teeth were acid etched by 37% phosphoric acid (Etching Gel, 3M Unitek, Monrovia, CA, USA) for 30 seconds, water rinsed for 10 seconds, and then dried for 20 seconds with oil-free compressed air until a chalky surface appeared. In this study, standard 18-slot edgewise brackets (Equilibrium Dentaurum Inc., Ispringen, Germany) were used.

After surface preparation, bonding process was carried out for each group based on one of the following protocols:

G1 (control): Assure primer (Reliance Orthodontic Product, Itasca, IL, USA) was applied to freshly etched enamel surface with a microbrush. Then, the enamel surface was gently air-dried for five seconds. A small amount of Assure adhesive (Reliance Orthodontic Product, Itasca, IL, USA) was squeezed into the mesh on the back of each bracket and was then pressed on the tooth surface. The excess adhesive material was removed and it was light-cured by LED light curing unit (Smart Life IQ2, Dentsply-Milford, USA**)** at 400nm wavelength for 40 seconds (10 seconds at each direction).

G2 (contaminated after etching): Prior to the primer application, the teeth were contaminated with saliva. Unstimulated human saliva was collected from one of the authors who had not consumed any food or liquids for 60 minutes prior to the collection [10, 11].

The rest of the procedure was the same as in G1.

G3 (contaminated after priming): Assure primer was applied to freshly etched enamel surface and gently air-dried for five seconds. Then, fresh saliva was applied with a microbrush on the labial surface until they were totally contaminated. The rest of the procedure was the same as in G1 and G2.

Fig. 1. Teeth sealed with wax

G4 (control): The teeth in this group were treated as in G1 except that the Moisture Intensive Primer Transbond (TMIP, 3M Unitek, Monrovia, CA, USA) and Transbond Plus Color Change Adhesive were used.
G5 (contaminated after etching): The teeth in this group were treated as in G2 except that Transbond Plus was used.
G6 (contaminated after priming): The teeth in this group were treated as in G3 except that the TMIP and Transbond Plus were used. Bonding procedures are summarized in Table 1. After the bonding procedure, the samples were stored in distilled water for four weeks. All samples were thermocycled for 500 cycles between 5°C and 55°C with 30 seconds of dwell time and 10 seconds of transfer time. Dye penetration method was used to evaluate the microleakage scores.
Following thermocycling, root apices of the teeth were sealed using sticky wax; the entire teeth surfaces were coated with nail polish except for 1 mm rim of tooth structure around the bracket (Fig. 1).
The teeth were stored in 2% methylene blue solution for 24 hours at room temperature. They were then cleaned by a toothbrush under running water, dried by air; and mounted in blocks containing auto-polymerizing acrylic resin. Four parallel longitudinal sections were made using low-speed diamond saw (Auccutom-50, Struers, Denmark) in buccolingual direction.
Dye penetration at the bracket/adhesive and enamel/adhesive interfaces was evaluated by a blinded tester under a stereomicroscope (Olympus SZX7, Olympus Optical, Tokyo, Japan) at ×16 magnification (Figs. 2 and 3).

Table 1. Bonding procedure of study groups

Groups	Contamination before priming	Primer	Contamination after priming	Adhesive
1	-	Assure	-	Assure
2	Saliva	Assure	-	Assure
3	-	Assure	Saliva	Assure
4	-	TMIP	-	Transbond Plus
5	Saliva	TMIP	-	Transbond Plus
6	-	TMIP	Saliva	Transbond Plus

Fig. 2. No micro leakage on none of the surfaces

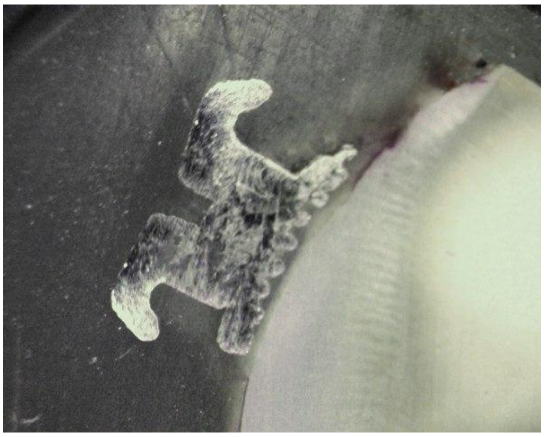

Fig. 3. Microleakage of enamel-adhesive surface

According to Arhun et al, [12] dye penetration at both incisal and gingival margins of the brackets at the bracket-adhesive and the adhesive-enamel interfaces was scored using a digital caliper (Shoka Golf, Tokyo, Japan). The data were recorded to the nearest value in the range of 0.5–5 mm. Scoring was done according to the following criteria: 0: no dye penetration at the bracket-adhesive or the adhesive-enamel interface; 1: dye penetration restricted to 1 mm at the bracket-adhesive or the adhesive-enamel interface; 2: dye penetration into the inner half (2 mm) of the bracket-adhesive or adhesive-enamel interface; 3: dye penetration into 3 mm of the bracket-adhesive or adhesive-enamel interface.
To determine the measurement error, two researchers examined the samples. Inter and intra-examiner kappa scores of both observers were over 0.75. In case of disagreement, the examiners discussed the case and agreed on a final score.
The frequency of each score at the two sides (occlusal and gingival) and surfaces (enamel-adhesive and enamel-bracket) of each group was demonstrated in the frequency table. Statistical analyses were performed using Fisher's exact test. In multiple comparisons, the Bonferroni correction was used to determine the critical value. Statistical significance level was P= 0.05.

RESULTS

Descriptive table (Table 2) shows the microleakage of the study groups at occlusal and gingival margins in both interfaces of adhesive. All samples showed some degrees of microleakage. The microleakage score in all samples was higher at gingival margins of both adhesive interfaces compared to the occlusal margins; although it was not statistically significant.
Multiple comparison of the microleakage scores of all the six study groups is shown in Table 3. When comparing the microleakage score at the enamel/adhesive interface, samples contaminated with saliva showed greater microleakage than control groups and the highest microleakage score was observed in G2 and G3, which were bonded with Assure and contaminated with saliva. This difference was statistically significant (P<0.01).
The results showed that the microleakage score at the adhesive-bracket interface was not significantly different between the groups. Comparison of the microleakage scores among the six study groups is shown inTable 3. In all contaminated groups, microleakage score at the enamel-adhesive interface (in both gingival and occlusal margins) was significantly higher than that at the adhesive-bracket interface (P< 0.01). In dry conditions, no significant difference was observed between Assure and TMIP.

Table 2. Descriptive statistics and comparison of the microleakage scores between occlusal and gingival sides at the enamel-adhesive and adhesive-bracket interfaces

Interface \ Side	Groups*	N	Occlusal score (frequency)				Gingival score (frequency)				Result	
			0	1	2	3	0	1	2	3	P value	Significance
Enamel Adhesive	1	15	5	10	0	0	4	10	1	0	1.00	NS
	2	15	0	5	5	5	0	3	8	4	0.635	NS
	3	15	0	6	6	3	0	4	4	7	0.45	NS
	4	15	8	7	0	0	6	9	0	0	0.715	NS
	5	15	3	10	2	0	2	9	4	0	0.762	NS
	6	15	4	9	2	0	1	11	3	0	0.536	NS
Adhesive bracket	1	15	7	8	0	0	5	10	0	0	0.71	NS
	2	15	5	9	1	0	2	10	3	0	0.322	NS
	3	15	6	8	1	0	3	10	2	0	0.547	NS
	4	15	9	5	1	0	7	8	0	0	0.448	NS
	5	15	8	6	1	0	5	10	0	0	0.274	NS
	6	15	7	8	0	0	5	8	2	0	0.378	NS

N=Number; NS=Non significant
*Group 1=Assure primer+Assure adhesive; Group 2= Saliva+Assure primer+Assure adhesive; Group 3=Assure primer+saliva+Assure past; Group 4= TMIP+Transbond Plus. Group 5=saliva+TMIP+Transbond Plus; group 6= TMIP+ saliva+Transbond Plus

Table 3. Multiple comparisons of the microleakage scores among the groups for occlusal and gingival sides at the enamel-adhesive and adhesive-bracket interface

Interface	Side	Groups*	N	Microleakage score (frequency)				P value	Multiple comparisons (P value)					Critical value
				0	1	2	3		2	3	4	5	6	
Enamel adhesive	Occlusal	1	15	5	10	0	0	.000	0.000	0.000	0.7	0.34	0.281	0.01
		2	15	0	5	5	5			0.34	0.000	0.01	0.003	0.0125
		3	15	0	6	6	3				0.000	0.008	0.006	0.015
		4	15	8	7	0	0					0.07	0.026	0.025
		5	15	3	10	2	0						0.75	0.05
		6	15	4	9	2	0							
	Gingival	1	15	4	10	1	0	.000	0.000	0.000	0.226	0.441	0.542	0.01
		2	15	0	3	8	4			0.806	0.02	0.01	0.008	0.0125
		3	15	0	4	4	7				0.000	0.023	0.017	0.015
		4	15	6	9	0	0					0.292	0.352	0.025
		5	15	2	9	4	0						1.00	0.05
		6	15	1	11	3	0							
Adhesive bracket	Occlusal	1	15	7	8	0	0	.889	0.158	0.442	0.71	1.00	0.607	0.01
		2	15	5	9	1	0			1.00	0.062	0.15	0.59	0.0125
		3	15	6	8	1	0				0.146	0.438	0.845	0.015
		4	15	9	5	1	0					0.710	0.478	0.025
		5	15	8	6	1	0						0.61	0.05
		6	15	7	8	0	0							
	Gingival	1	15	5	10	0	0	.353	0.705	1.00	0.464	0.718	1.00	0.01
		2	15	2	10	3	0			1.00	0.35	0.71	0.71	0.0125
		3	15	3	10	2	0				0.711	0.847	1.00	0.015
		4	15	7	8	0	0					1.00	0.466	0.025
		5	15	5	10	0	0						0.71	0.05
		6	15	5	8	2	2							

N=Number,
*Group 1= Assure primer+ Assure adhesive; Group 2= Saliva+ Assure primer+ Assure adhesive; Group 3= Assure primer+ saliva+ Assure past; Group 4= TMIP+ Transbond Plus. Group 5= Saliva+ TMIP+ Transbond Plus; Group 6= TMIP+ saliva+ Transbond Plus

DISCUSSION

In restorative dentistry, passage of fluids and molecules through the tooth-restoration interface is called microleakage, which cannot be diagnosed clinically and may cause secondary caries and post-operative tooth hypersensitivity [13]. In orthodontics, microleakage is defined as the passage of liquids and particles through the tooth-adhesive and adhesive-bracket interfaces, which may cause enamel decalcification, corrosion, discoloration and decreased bond strength [3]. White spot lesions were found in a quarter of patients treated with fixed orthodontic appliances [14].

Water [15,16] and saliva [15,17] can significantly reduce the bond strength between bracket and enamel and are reported to be the most common reasons for bond failure.

In an attempt to overcome this problem, hydrophilic bonding systems such as Transbond Plus Color Change and Assure were introduced. These products have been proven to be moisture resistant and can acceptably bond to saliva-contaminated enamel surfaces [10,11].

In the current study, the microleakage score of brackets bonded with the above-mentioned two hydrophilic bonding systems was measured in presence or absence of saliva contamination using dye penetration method [3,12,18,19]. To determine the measurement error, two observers assessed the samples. Inter and intra-examiner kappa scores of both observers were above 0.75.

The results showed that microleakage score at the gingival margin of all samples was higher than that at the occlusal margin, although it was not significant. This finding is consistent with previous studies [12,18, 20]. Arhun et al. [12] reported that the premolar teeth morphology is the main reason of this finding. Thicker adhesive on the gingival side, due to prominent buccal contour can cause higher microleakage score at this area. Also, Ramoglu et al. [21] believed that thinner adhesive on the occlusal side was the reason for lower microleakage score at this side.

In dry conditions, no significant differences were observe between microleakage scores of the two adhesive systems in the two sides and the two interfaces, which is similar to the results of previous studies. Arhun et al, [12] Alkis et al, [18] and Yagci et al. [22] concluded that the amount of microleakage was not affected by the type of adhesive.

In this study, all groups except for G1 and G4 (control groups) showed significantly higher microleakage at the enamel-adhesive interface at both occlusal and gingival sides compared with bracket-adhesive interfaces. Being closer to saliva at the enamel–adhesive interface rather than the bracket-adhesive interface might be a possible reason.

In the current study, brackets bonded with both bonding systems, when contaminated immediately after etching, showed higher microleakage compared with those contaminated after priming although the difference was not statistically significant. The reason has been explained by several studies. Saliva contamination after etching causes the water and glycoproteins in the saliva to interfere with proper adhesion. When surfaces are contaminated with saliva after application of primer and adhesive, saliva can affect the degree of conversion and bond strength; because hydroxyethyl methacrylate (HEMA) molecules with their hydrophilic nature may retain water within the adhesive layer; thus, they can no longer participate in chain growth during polymerization [23-26].

The results showed that microleakage scores at the enamel-adhesive interface at both gingival and occlusal sides were lower for Transbond Plus/TMIP adhesive system in wet conditions ($P<0.05$). The reason for better performance of Transbond Plus adhesive in wet conditions may be its chemical properties.

TMIP primer like other moisture resistant primers is produced by dissolving hydrophilic primers in ethanol or acetone.

A reversible hydrolytic bond, beside micromechanical retention, is created by breaking and reforming of carboxylate salt complexes between ionized carboxyl groups of primer and calcium of enamel. This phenomenon may improve the quality of moisture resistance.

The polyethylene glycol dimethacrylate (PEGDMA) is one of the Transbond Plus Color Chang adhesive components, which might be another factor improving its resistance in wet environment. The lower bisphenol A diglycidyl ether dimethacrylate (bis-GMA) content (>2%) in this adhesive, compared with traditional adhesives e.g. Transbond XT (10-20%), resulted in higher proportional content of PEGDMA that might enhance the infiltration of bis-GMA adhesive into moistened enamel [27].

Percolation phenomenon is another factor associated with increased microleakage. Thermocycling was performed to consider the unmatched coefficients of thermal expansion of adhesive material and tooth substance [3,12]. Thermocycling was not performed in some recent studies because it was assumed to be ineffective on microleakage [20,21]. However, Vicente et al. [28] reported that bovine incisors bonded with Transbond XT adhesive and thermocycled showed significantly higher microleakage score at the enamel-adhesive interface. This might be an explanation for the higher microleakage scores found in this study compared to previous studies [20,21]. Several studies reported that there was a correlation between the microleakage score at the bracket-adhesive interface and bond strength [19,20]. However, James et al. [29] did not find such a correlation. Short-term evaluation of microleakage was a limitation of this study while in clinical conditions, brackets remain in the mouth for a long time (about two years).

The small sample size was another limitation of the current study, which might affect the generalizability of the findings; therefore, we recommend further clinical studies with larger sample sizes and longer evaluation periods to investigate the effect of saliva contamination during the bonding process on microleakage.

CONCLUSION

In dry conditions, no significant difference was observed between the Assure and TMIP. Enamel-adhesive interface showed a higher microleakage score following saliva contamination compared to bracket-adhesive interface. In saliva contaminated groups, lower microleakage score was observed at the enamel-adhesive interface of Transbond Plus/TIMP compared to Assure.

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