ORIGINAL ARTICLE

Effect of blood and saliva contamination on shear bond strength of brackets bonded with 4 adhesives

Mehmet Oğuz Öztoprak,[a] Fulya Isik,[b] Korkmaz Sayınsu,[b] Tulin Arun,[c] and Bulent Aydemir[a]
Istanbul, Turkey

Introduction: The purpose of this study was to assess the effect of blood and saliva contamination on the shear bond strength of 4 orthodontic adhesives. **Methods:** Four adhesives (Transbond XT primer [3M Unitek, Monrovia, Calif], Transbond Plus self-etch primer [3M Unitek], Assure hydrophilic primer [Reliance, Itasca, Ill], and SmartBond cyanoacrylate [Gestenco, Gothenburg, Sweden]) were used to bond stainless steel maxillary central incisor brackets to 120 bovine permanent mandibular incisors. The teeth were randomly divided into 12 groups of 10 specimens, and each primer-adhesive combination was tested under different enamel conditions: dry, and blood and saliva contamination after priming. Shear forces were applied to the samples with a testing machine. Bond strengths were measured in megapascals. **Results:** Statistical evaluations showed that the shear bond strength of the SmartBond cyanoacrylate adhesive group was significantly lower than all other groups; however, it was the only adhesive that was not affected by contamination. Saliva and blood contamination resulted in significant drops in shear bond strengths of the Transbond XT and Assure groups. Transbond Plus self-etch primer was also negatively affected by blood contamination, although it was suitable for bonding with saliva contamination. (Am J Orthod Dentofacial Orthop 2007;131:238-42)

Etching tooth surfaces with phosphoric acid to bond acrylic resin to tooth enamel was introduced in 1955 by Buonocore.[1] Acid etching differentially dissolves enamel crystals in the prism structure; this results in a roughened surface ready for micromechanical retention. Traditional composite resin bonding materials require completely dry surfaces to obtain clinically acceptable bond strength. However, various clinical conditions do not permit ideal isolation in the bonding site, especially when bonding attachments to hard-to-reach places near the gingival area and around second molars, or when exposing and attaching buttons to partially erupted or impacted ectopic teeth.[2-5]

When etched enamel becomes wet, most of the porosities become plugged, and resin penetration is impaired. This results in resin tags of insufficient numbers and lengths. It is suggested that even momentary saliva or blood contamination adversely affects the bond, because saliva and blood deposit organic adhesive coatings that resist washing in the first few seconds of exposure.[6-7] Previous studies that evaluated the effect of blood contamination on the bond strengths of light-cured composites showed significant reductions in bond strength values.[3,8] To address this problem, manufacturers introduced hydrophilic bonding materials, suggesting the possibility of obtaining successful orthodontic bonding to a moisture-contaminated enamel surface.

The hydrophilic resin system Assure (Reliance, Itasca, Ill) was tested under saliva contamination, with the results pointing to adequate, clinically acceptable bond strength.[9-11] Another material, Transbond Plus self-etch primer (SEP) (3M Unitek, Monrovia, Calif) was reported to be unaffected by water or saliva contamination because of its inherent hydrophilic properties.[4,7,11,12] In another study, Sfondrini et al[13] tested the bond strength for Transbond Plus SEP with blood contamination and concluded that it significantly reduced bond strength values.

More recently, a new cyanoacrylate adhesive—SmartBond (Gestenco, Gothenburg, Sweden)—was introduced that did not require the use of a primer or a curing light during bonding. The adhesive is activated when it comes into contact with a wet tooth surface. Several studies compared the bond strength of the cyanoacrylate adhesive with other conventional adhe-

From the Faculty of Dentistry, Yeditepe University, Istanbul, Turkey.
[a]Research assistant.
[b]Assistant professor.
[c]Professor.
Reprint requests to: Dr Fulya Isik, Faculty of Dentistry, Yeditepe University, Bagdat Cad. No: 238 Goztepe 34728, Istanbul, Turkey; e-mail, drfulya@yeditepe.edu.tr.
Submitted, November 2004; revised and accepted, February 2005.
0889-5406/$32.00

doi:10.1016/j.ajodo.2005.02.035

Table I. Bonding procedures for different enamel surface conditions

Group 1	37% phosphoric acid	rinsing/drying	dry	Transbond XT	light-curing
Group 2	37% phosphoric acid	rinsing/drying	blood	Transbond XT	light-curing
Group 3	37% phosphoric acid	rinsing/drying	saliva	Transbond XT	light-curing
Group 4	SEP		dry	Transbond Plus	light-curing
Group 5	SEP		blood	Transbond Plus	light-curing
Group 6	SEP		saliva	Transbond Plus	light-curing
Group 7	35% phosphoric acid	rinsing/wet		SmartBond	
Group 8	35% phosphoric acid	rinsing/wet	blood	SmartBond	
Group 9	35% phosphoric acid	rinsing/wet	saliva	SmartBond	
Group 10	37% phosphoric acid	rinsing/drying	dry	Assure	light-curing
Group 11	37% phosphoric acid	rinsing/drying	blood	Assure	light-curing
Group 12	37% phosphoric acid	rinsing/drying	saliva	Assure	light-curing

sives.[14-17] All found small but clinically acceptable bond strengths.

These studies of bond strengths with blood and saliva contamination need updating because new materials are always being introduced. The purpose of our study was to investigate the bond strengths of 4 bonding materials, Transbond XT primer (3M Unitek), Transbond Plus SEP, Assure hydrophilic primer, and SmartBond cyanoacrylate adhesive, with blood and saliva contamination. The null hypothesis was that there is no significant difference in bond strengths of brackets bonded with the various materials under dry conditions, and saliva and blood contamination.

MATERIAL AND METHODS

One hundred twenty freshly extracted bovine permanent mandibular incisors were collected, cleaned of soft tissue, and stored in a solution of 70% (weight/volume) ethyl alcohol. The criteria for tooth selection included intact buccal enamel, no pretreatment chemical agents (eg, hydrogen peroxide), no cracks caused by the extraction forceps, and no caries. The teeth were cleaned and then polished with pumice and rubber prophylactic cups for 10 seconds.

The teeth were randomly assigned to 12 groups. Each group consisted of 10 specimens. One hundred twenty stainless steel maxillary central incisor brackets with a 0.018-in slot (DynaLock, 3M Unitek) were bonded by 1 operator (M.O.Ö.).

Three enamel surface conditions were studied: dry, contaminated with blood, and contaminated with saliva. For each enamel surface condition, 4 orthodontic adhesive systems were used: Transbond XT, Transbond plus SEP, Assure hydrophilic primer, and SmartBond cyanoacrylate adhesive. The bonding procedure for each group is described in Table I.

The teeth in groups 1, 2, and 3 were conditioned with 37% phosphoric acid for 15 seconds followed by thorough washing and drying. A thin coat of Transbond XT primer was applied, and the brackets were bonded with Transbond XT resin and light-cured with a halogen light-curing unit (Optilux, Kerr, Orange, Calif) for 20 seconds on the mesial side and 20 seconds on the distal side (total cure time, 40 seconds).

The teeth in groups 4, 5, and 6 were conditioned with Transbond Plus SEP, which contains both etchant and primer. For activation, the 2 components were squeezed together, and the resulting mix was applied directly on the tooth surface, rubbed for 3 seconds, and then gently evaporated with an oil-free air burst, according to the manufacturer's instructions. The brackets were bonded with Transbond XT resin and light-cured with a halogen light-curing unit for 20 seconds on the mesial side and 20 seconds on the distal side (total cure time, 40 seconds).

The teeth in groups 7, 8, and 9 were conditioned with 35% phosphoric acid for 15 seconds followed by thorough washing and drying. A moist cotton roll was used to wet the enamel surface before the adhesive was applied. Once the SmartBond adhesive came into contact with the wet enamel surface, the clinician had 3 to 5 seconds to adjust the placement of the bracket before the adhesive starts to set within 3 to 5 minutes.

The teeth in groups 10, 11, and 12 were conditioned with 37% phosphoric acid for 15 seconds followed by thorough washing and drying. A thin coat of Assure primer was applied, and the brackets were bonded with Transbond XT resin and light-cured with a halogen light-curing unit for 20 seconds on the mesial side and 20 seconds on the distal side (total cure time, 40 seconds).

To achieve reproducible conditions, the teeth in groups 2, 5, 8, and 11 were contaminated with fresh human blood from a male donor; the blood was applied with a brush on the labial surfaces until they were totally contaminated. The teeth in groups 3, 6, 9, and 12 were contaminated with human saliva from a male donor, who was instructed to brush his teeth and not to

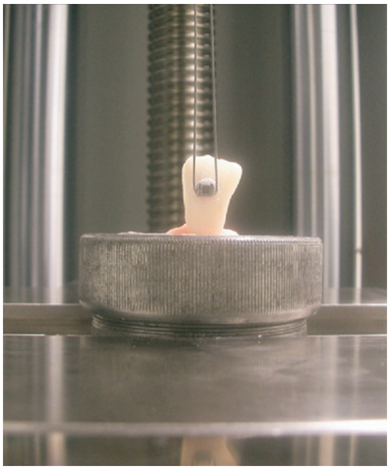

Fig 1. Specimen in testing machine.

eat for 1 hour before the saliva was collected. Saliva was applied with a brush on the labial surfaces until they were totally contaminated.

After bonding, all samples were stored in distilled water at 37°C for 72 hours. Each tooth was oriented with a guiding device, so that its labial surface was parallel to the force during the shear strength test. Then the specially prepared cylindrical metal ring was placed around the tooth. The ring was filled with self-curing, fast-setting acrylic to 3 mm below the bracket. A 0.016 × 0.022-in stainless steel wire was placed under the wings of bracket with ends of the wire clamped to the self-centering upper jaw of the universal testing machine (Zwick, Ulm, Germany) (Fig 1). The specimen was stressed in a gingivo-occlusal direction to failure with a speed of 3 mm per minute. A computer electronically connected to the testing machine recorded the results of each test. Bond strengths were measured in megapascals (MPa).

Statistical calculations were performed with the GraphPad Prisma version 3.0 software (San Diego, Calif) for Windows. In addition to standard descriptive statistical calculations (mean and standard deviation), the nonparametric Kruskal-Wallis test was carried out to compare the adhesive groups. In the evaluation of subgroups, the Dunn multiple comparison test was performed. The results were evaluated with a 95% confidence interval. The statistical significance level was established at $P < .05$.

RESULTS

The means and standard deviations in all groups are shown in Table II and Figure 2. When the results were statistically evaluated, the shear bond strengths of the SmartBond cyanoacrylate adhesive group were significantly lower than those for the other groups (Table III); however, it was the only adhesive that was not affected by contamination conditions. Saliva and blood contamination resulted in significant drops in shear bond strengths of the Transbond XT and Assure groups (Table IV). Transbond Plus SEP was also affected by blood contamination, even though it was a suitable adhesive for bonding with saliva contamination.

DISCUSSION

In this study, 4 orthodontic adhesives (Transbond XT primer, Transbond Plus SEP, Assure hydrophilic primer, and SmartBond cyanoacrylate adhesive) were evaluated with 3 surface conditions (dry, and blood and saliva contamination).

Previous studies showed that bovine and human enamel are similar in their physical properties, compositions, and bond strengths.[4,5,8,9,18] Bovine enamel was reported to be a reliable substitute for human enamel in bonding studies.[18] Thus, we used bovine mandibular incisors because they were readily available and inexpensive, and had a close morphological similarity to human enamel.

Acid etching was done for 15 seconds as recommended by the manufacturers. Olsen et al[19] also concluded that clinically acceptable bond strengths could be obtained with etching times as short as 10 seconds. Each bracket was light-cured for 40 seconds (20 seconds on the mesial side and 20 seconds on the distal side) as recommended by Oesterle et al,[20] instead of the 20 seconds recommended by 3M Unitek for Transbond XT primer.

Contamination by oral fluids such as saliva and plasma was reported to reduce the bond strength of direct bonding of adhesive to enamel.[21] Saliva and blood behave differently because of differences in the types and amounts of inorganic and organic substances they contain.[22] In this study, the null hypothesis was rejected. Uncontaminated enamel surfaces had the highest bond strengths for both conventional (Transbond XT) and hydrophilic (Assure) primers. Among all blood-contaminated conditions, Assure had significantly higher shear bond strength values than did Transbond XT. The bond strength of Transbond XT (group 1) at 15.28 MPa was significantly greater than the bond strengths of the Transbond XT blood- and saliva-contaminated groups (3.08 and 3.79 MPa, respectively). These findings agreed with previous studies.[4,9,13,23] In all blood- and saliva-contaminated groups, Transbond XT showed the weakest bond strength values. There was no significant difference between Transbond XT primer (group 1), Transbond

Table II. Comparison of shear bond strengths of groups (in MPa)

	Control (n = 10)		Blood (n = 10)		Saliva (n = 10)		
	Mean	SD	Mean	SD	Mean	SD	P
Transbond XT	15.28	1.96	3.08	1.81	3.79	2.20	<.0001
Transbond Plus SEP	13.76	2.76	5.28	1.47	13.80	3.96	<.0001
SmartBond	5.66	2.51	6.14	0.95	5.85	1.79	<.05
Assure	16.40	3.50	6.83	2.88	10.66	1.67	<.0001
P	<.0001		<.01		<.0001		

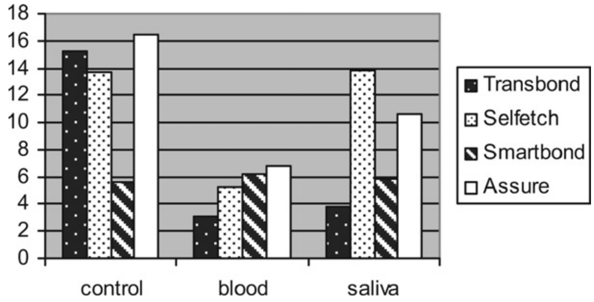

Fig 2. Shear bond strengths of groups.

Table III. Comparison (*P* values) of bonding materials with respect to contamination groups

Dunn multiple comparison test	Control	Blood	Saliva
Transbond XT/Transbond Plus SEP	<.05	>.05	<.001
Transbond XT/SmartBond	<.001	<.01	>.05
Transbond XT/Assure	>.05	<.01	<.001
Transbond plus SEP/SmartBond	<.01	>.05	<.001
Transbond plus SEP/Assure	>.05	>.05	>.05
SmartBond/Assure	<.001	>.05	<.05

Plus SEP (group 4), and Assure primer (group 10) in dry environments.

In the group bonded with Transbond Plus SEP, saliva contamination did not significantly decrease the shear bond strength of the orthodontic brackets. There was no significant difference in shear bond strength of Transbond Plus SEP in the dry environment (13.76 MPa) and contaminated with saliva (13.80 MPa), but blood contamination (group 5) resulted in a significant drop in the shear bond strength (5.28 MPa). These findings agreed with previous studies.[2,5,11,12,24,25] In this study, the reason for the higher bond strength values in dry and blood-contaminated conditions with Transbond XT, when compared with the study by Sfondrini et al,[13] might be the result of different durations of light-curing (20 seconds). On the other hand, our bond strength results with Transbond Plus SEP on dry and saliva-contaminated tooth surfaces were lower than those of Cacciafesta et al,[5] but their tooth and bonding agent combinations were different from our study.

Table IV. Comparison (*P* values) of groups

Dunn multiple comparison test	Transbond	Transbond Plus SEP	SmartBond	Assure
Control/blood	<.001	<.001	—	<.001
Control/saliva	<.01	>.05	—	<.05
Blood/saliva	>.05	<.01	—	<.05

Between the groups bonded with SmartBond cyanoacrylate adhesive, there were no significant differences in the bond strengths of wet, and blood- and saliva-contaminated conditions. The shear bond strengths of SmartBond were significantly lower in the control group than all other adhesives. In this study, the use of the cyanoacrylate adhesive to bond orthodontic brackets to enamel surface provided the clinically acceptable bond force levels (5.9-7.8 MPa) suggested by Reynolds.[26] Our results for SmartBond are parallel with most other studies[15-17] except that of Örtendahl et al.[14] The higher bonding values reported by these authors could arise from the differences in tooth material.

According to the manufacturer, Assure Universal bonding agent bonds to normal, atypical, dry, or slightly contaminated enamel, and it can be used with any light- or chemical-curing system. Our data showed that Transbond XT adhesive combined with Assure hydrophilic primer in a dry environment had the greatest bond strength (16.4 MPa) of all the test groups. The bond strengths of the Assure blood-contaminated (6.83 MPa) and Assure saliva-contaminated (10.66 MPa) groups were significantly lower than Assure in the dry environment. Other studies carried out with the same bonding agent had similar[10] or greater bond strengths.[9]

CONCLUSIONS

1. Transbond XT adhesive used with Transbond XT primer and Assure hydrophilic primer in a dry environment, and Transbond plus SEP in dry and

saliva-contaminated environments showed the highest bond strengths of the test groups.

2. Transbond XT adhesive used with Transbond XT primer yielded the weakest bonding strength of the groups with saliva and blood contamination.
3. SmartBond cyanoacrylate adhesive showed similar low bond strengths in all conditions.
4. Saliva had no effect on the bond strength of Transbond XT adhesive bonded with Transbond Plus SEP. The second-best alternative with saliva contamination was Transbond XT adhesive bonded with Assure primer.
5. Under blood-contaminated conditions, both Assure hydrophilic primer with Transbond XT adhesive and SmartBond, even though they produced significantly lower bond strengths than control conditions, had significantly higher bond strength values than the conventional primer and adhesive combination.

REFERENCES

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34: 849-53.
2. Dorminey JC, Dunn WJ, Taloumis LJ. Shear bond strength of orthodontic brackets bonded with a modified 1-step etchant-and-primer technique. Am J Orthod Dentofacial Orthop 2003;124: 410-3.
3. Hobson RS, Ledvinka J, Meechan JG. The effect of moisture and blood contamination on bond strength of a new orthodontic bonding material. Am J Orthod Dentofacial Orthop 2001;120: 54-7.
4. Cacciafesta V, Sfondrini MF, De Angelis M, Scribante A, Klersy C. Effect of water and saliva contamination on shear bond strength of brackets bonded with conventional, hydrophilic, and self-etching primers. Am J Orthod Dentofacial Orthop 2003;123: 633-40.
5. Cacciafesta V, Sfondrini MF, Baluga L, Scribante A, Klersy C. Use of a self-etching primer in combination with resin-modified glass ionomer: effect of water and saliva contamination on shear bond strength. Am J Orthod Dentofacial Orthop 2003;124:420-6.
6. Hormati AA, Fuller JL, Denehy GE. Effects of contamination and mechanical disturbance on the quality of acid-etched enamel. J Am Dent Assoc 1980;100:34-8.
7. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. J Am Dent Assoc 1985;110:329-32.
8. Cacciafesta V, Sfondrini MF, Scribante A, De Angelis M, Klersy C. Effects of blood contamination on the shear bond strengths of conventional and hydrophilic primers. Am J Orthod Dentofacial Orthop 2004;126:207-12.
9. Webster MJ, Nanda RS, Duncanson MG Jr, Khajotia SS, Sinha PK. The effect of saliva on shear bond strengths of hydrophilic bonding systems. Am J Orthod Dentofacial Orthop 2001;119: 54-8.
10. Schaneveldt S, Foley TF. Bond strength comparison of moisture-insensitive primers. Am J Orthod Dentofacial Orthop 2002;122: 267-73.
11. Zeppieri IL, Chung CH, Mante FK. Effect of saliva on shear bond strength of an orthodontic adhesive used with moisture-insensitive and self-etching primers. Am J Orthod Dentofacial Orthop 2003;124:414-9.
12. Rajagopal R, Padmanabhan S, Gnanamani J. A comparison of shear bond strength and debonding characteristics of conventional, moisture-insensitive, and self-etching primers in vitro. Angle Orthod 2004;74:264-8.
13. Sfondrini MF, Cacciafesta V, Scribante A, De Angelis M, Klersy C. Effect of blood contamination on shear bond strength of brackets bonded with conventional and self-etching primers. Am J Orthod Dentofacial Orthop 2004;125:357-60.
14. Örtendahl TW, Mohlin B, Örtengren U. A new orthodontic bonding adhesive. J Clin Orthod 2000;34:50-4.
15. Bishara SE, VonWald L, Laffoon JF, Warren JJ. Effect of using a new cyanoacrylate adhesive on the shear bond strength of orthodontic brackets. Angle Orthod 2001;71:466-9.
16. Bishara SE, Laffoon JF, VonWald L, Warren J. Effect of time on the shear bond strength of cyanoacrylate and composite orthodontic adhesives. Am J Orthod Dentofacial Orthop 2002;121: 297-300.
17. Ajlouni R, Bishara SE, Oonsombat C. Effect of water storage on the shear bond strength of a cyanoacylate adhesive clinical implications. World J Orthod 2004;5:250-3.
18. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop 1998;113:514-9.
19. Olsen ME, Bishara SE, Boyer DB, Jacobsen JR. Effect of varying etching times on the bond strength of ceramic brackets. Am J Orthod Dentofacial Orthop 1996;109:403-9.
20. Oesterle LJ, Messersmith ML, Devine SM, Ness CF. Light and setting times of visible light cured orthodontic adhesives. J Clin Orthod 1995;29:31-6.
21. Sirirungrojying S, Saito K, Hayakawa T, Kasai K. Efficacy of using self-etching primer with a 4-META/MMA-TBB resin cement in bonding orthodontic brackets to human enamel and effect of saliva contamination on shear bond strength. Angle Orthod 2004;74:251-8.
22. Itoh T, Fukushima T, Inoue Y, Arita S, Miyazaki K. Effect of water saliva and blood contamination on bonding of metal brackets with a 4-META/MMA/TBB resin to etched enamel. Am J Dent 1999;12:299-304.
23. Evrenol Bİ, Arun T, Küçükkeleş N. Comparison of shear bond strength of fluoride releasing materials. J Clin Pediatr Dent 1999;23:315-9.
24. Büyükyılmaz T, Üşümez S, Karaman AI. Effect of self-etching primers on bond strength—are they reliable? Angle Orthod 2003; 73:64-70.
25. Grubisa HS, Heo G, Raboud D, Glover KE, Major PW. An evaluation and comparison of orthodontic bracket bond strengths achieved with self-etching primer. Am J Orthod Dentofacial Orthop 2004;126:213-9.
26. Reynolds IR. A review of direct orthodontic bonding. Br J Orthod 1979;2:171-8.