Effects of blood contamination on the shear bond strengths of conventional and hydrophilic primers

Vittorio Cacciafesta, DDS, MSc, PhD,^a Maria Francesca Sfondrini, MD, DDS,^b Andrea Scribante, DDS,^c Marco De Angelis, DDS,^c and Catherine Klersy, MD, MSc^d

Pavia, Italy

The purpose of this study was to assess the effect of blood contamination on the shear bond strength and failure site of 2 orthodontic primers (Transbond XT and Transbond MIP; 3M/Unitek, Monrovia, Calif) when used with adhesive-precoated brackets (APC II brackets; 3M/Unitek). One hundred twenty bovine permanent mandibular incisors were randomly divided into 8 groups; each group contained 15 specimens. Each primer-adhesive combination was tested under a different enamel surface condition: dry, blood contamination before priming, blood contamination after priming, or blood contamination before and after priming. Stainless steel APC II brackets were bonded to the teeth. After bonding, all samples were stored in distilled water at room temperature for 24 hours and subsequently tested for shear bond strength. Noncontaminated enamel surfaces had the highest bond strengths for both conventional and hydrophilic primers; their values were almost the same. Under blood-contaminated conditions, both primers showed significantly lower shear bond strengths. For each type of primer, no significant differences were reported among the bloodcontaminated groups. Significant differences in debond locations were found among the groups bonded with the 2 primers under the various enamel surface conditions. Blood contamination of enamel during the bonding procedure of conventional and hydrophilic primers significantly lowers their bond strength values and might produce a bond strength that is not clinically adequate. (Am J Orthod Dentofacial Orthop 2004; 126:207-12)

B onding of orthodontic brackets with composite resin adhesives requires a dry field of operation. The properties of an adhesive resin can be diminished by various intraoral factors, that include high humidity in the oral cavity,^{1,2} aging of the tooth,³ dental caries,⁴ and saliva or blood contamination of the adhesive areas.^{1,5-8} When orthodontists and surgeons collaborate in the exposure and orthodontic alignment of unerupted ectopic teeth, it is difficult to work under ideal conditions. Treatment options include either exposure only of the tooth or exposure of the tooth and direct bonding of the appliance for orthodontic traction. Delaying the bonding procedure until healing results in

Copyright @ 2004 by the American Association of Orthodontists. doi:10.1016/j.ajodo.2003.06.022

little risk of contamination with blood or moisture.⁹ However, the soft tissues that cover the tooth must be excised or repositioned to expose its crown. This can result in a poor gingival margin.^{10,11} In such a situation, the only option is to bond the orthodontic appliance to the tooth at the time of operation. However, fluid contamination during bonding can lead to premature failure of the bond.¹²⁻¹⁵ 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.¹⁴ Even momentary saliva or blood contamination adversely affects the bond, because saliva and blood deposit an organic adhesive coating in the first few seconds of exposure that resists washing.¹⁵ Thus, it would be advantageous to successfully bond to enamel in a blood-contaminated environment, particularly on partially erupted and impacted teeth.

Previous studies that evaluated the effect of blood contamination on the bond strengths of light-cured composites showed a significant reduction in bond strength values.^{1,9}

To address this reality, manufacturers have introduced hydrophilic bonding materials, suggesting the

^aAssistant clinical professor, Department of Orthodontics, University of Insubria, Varese, Italy.

^bAssistant clinical professor, Department of Orthodontics, University of Pavia, Pavia, Italy.

^cResearch fellow, Department of Orthodontics, University of Pavia, Pavia, Italy.

^dStatistician, Clinical Epidemiology and Biometry Unit, Scientific Direction, IRCCS San Matteo, Pavia, Italy.

Reprint requests to: Dr Vittorio Cacciafesta, c/o Studio Prof Giuseppe Sfondrini, Via Libertà 17, 27100 Pavia, Italy; e-mail, vcacciafesta@hotmail.com. Submitted, April 2003; revised and accepted, June 2003. 0889-5406/\$30.00



Fig 1. Diagram of study's specimen grouping.

possibility of obtaining successful orthodontic bonding to a moisture-contaminated enamel surface. Some hydrophilic enamel primers for orthodontic treatment are formulated with alcohol or acetone to displace moisture from the isolated enamel surface.¹⁶ Transbond Moisture Insensitive Primer (Transbond MIP, 3M/Unitek, Monrovia, Calif) contains a hydrophilic primer dissolved in acetone and is recommended for use on dry or wet etched enamel in conjunction with either self-cured or light-cured bonding agents. Some authors found a significant bond strength reduction by using Transbond MIP in dry environments,¹⁷ but others reported no differences, in terms of bond strength, between conventional and moisture-insensitive primers applied to dry enamel.¹⁶

Enamel surface contamination can occur at 2 critical times: after the tooth surface has been etched and after the primer has been applied. Bonding could be compromised at both times.

Previous studies on the effect of moisture contamination on the resulting bond strength have evaluated hydrophilic primers (Transbond MIP) on dry versus water-contaminated,^{9,18,19} saliva-contaminated,^{16,18-20} and blood-contaminated enamel.⁹ However, in those studies, the contaminating agents were applied during just a single step of the bonding procedure, except for the study by Webster et al.¹⁶ So far, to our knowledge, no studies in the literature have evaluated the effects of blood contamination at different times of the bonding procedure on the bond strength values of hydrophilic primers.

Accordingly, the purpose of this study was to evaluate the effects of blood contamination (before priming, after priming, and before and after priming) on the shear bond strength and the site of bond failure of conventional and hydrophilic primers.

MATERIAL AND METHODS

One hundred twenty freshly extracted bovine permanent mandibular incisors were collected from a local slaughterhouse and stored in a solution of 0.1% (weight/volume) thymol (an antimicrobial to inhibit bacterial growth) for 1 week at 4°C. The criteria for tooth selection included intact buccal enamel with no cracks caused by extraction and no caries. The teeth were randomly assigned to 8 groups. Each group contained 15 specimens. The teeth were cleansed of soft tissue and embedded in cold-curing, fast-setting acrylic (Leocryl, Leone, Sesto Fiorentino, Italy). Metal rings (diameter, 15 mm) were filled with the acrylic resin and allowed to cure, thus encasing the specimen while allowing the buccal surface of enamel to be exposed. Each tooth was oriented so that its labial surface was parallel to the shearing force.

One hundred twenty adhesive-precoated stainlesssteel maxillary central-incisor brackets with 0.018-in slots (APC II brackets, 3M/Unitek, Monrovia, Calif) were bonded by 1 operator (M.D.A.). The average bracket-base surface area was reported by the manufacturer to be 11.7 mm². This was verified by measuring it with a digital caliper (Mitutoyo, Japan). The areas of 15 brackets were recorded, and the mean value was calculated.

Before bonding, the facial surface of each incisor was cleaned for 10 seconds with a mixture of water and fluoride-free pumice in a rubber polishing cup with a low-speed handpiece. The enamel surface was rinsed with water to remove pumice or debris and dried with an oil-free air stream.

Two orthodontic primers were studied: a conventional (Transbond XT, 3M/Unitek) and a hydrophilic (Transbond MIP, 3M/Unitek) primer. The orthodontic adhesive system used for bonding all brackets was the APC II adhesive (3M/Unitek), a modified version of Transbond XT (3M/Unitek). A diagram of the study design is given in Figure 1. Each primer-adhesive combination was tested under 4 different enamel surface conditions: dry, blood contamination before priming, blood contamination after priming, and blood contamination before and after priming. The bonding procedure for each treatment group is described in Table I.

Primer groups							
СР	1	etching drying		XT primer		bonding	light-curing
	2	etching drying	blood	XT primer	_	bonding	light-curing
	3	etching drying		XT primer	blood	bonding	light-curing
	4	etching drying	blood	XT primer	blood	bonding	light-curing
HP	1	etching drying		MIP	_	bonding	light-curing
	2	etching drying	blood	MIP	_	bonding	light-curing
	3	etching drying		MIP	blood	bonding	light-curing
	4	etching drying	blood	MIP	blood	bonding	light-curing

Table I. Bonding procedures for different surface conditions

CP, Conventional primer; HP, hydrophilic primer.

Teeth bonded with the conventional primer, Transbond XT, were etched with 37% phosphoric acid gel (3M/Unitek) for 30 seconds, followed by thorough washing and drying. Then the adhesive-precoated bracket was applied on the etched enamel near the center of the facial surface of the tooth with sufficient pressure to express excess adhesive, which was removed from the margins of the bracket base with a scaler before polymerization.

Teeth bonded with the hydrophilic primer, Transbond MIP, were etched with 37% phosphoric acid gel (3M Dental Products, Monrovia, Calif) for 30 seconds, followed by thorough washing and drying. After priming, the adhesive-precoated bracket was applied on the etched enamel near the center of the facial surface of the tooth with sufficient pressure to express excess adhesive, which was removed from the margins of the bracket base with a scaler before polymerization.

To achieve reproducible conditions, groups 2, 3, and 4 of the 2 primers were contaminated with fresh human blood from a male donor; it was applied with a brush onto the labial surfaces until they were totally contaminated (Table I).

All brackets were light-cured for 10 seconds on the mesial side and for 10 seconds on the distal side (total cure time 20 seconds) with a halogen light-curing unit (Ortholux XT, 3M/Unitek).

After bonding, all samples were stored in distilled water at room temperature for 24 hours and subsequently tested in a shear mode on a universal testing machine (Model 4301, Instron, Canton, Mass). The specimens were secured in the lower jaw of the machine so that the bonded bracket bases were parallel to the shear force direction. Specimens were stressed in an occluso-gingival direction at a crosshead speed of 1 mm/min, as in previous studies.²¹⁻²³ The maximum load necessary to debond or initiate bracket fracture was recorded in newtons and then converted into megapascals as a ratio of newtons to surface area of the bracket.

After bond failure, the bracket bases and the enamel surfaces were examined by the same operator (M.D.A.) under an optical microscope (Stereomicroscope SR, Zeiss, Oberkochen, Germany) at $10 \times$ magnification. The adhesive remnant index (ARI) was used to assess the amount of adhesive left on the enamel surface.²⁴ This scale ranges from 0 to 3. A score of 0 indicates no adhesive remaining on the tooth in the bonding area; 1 indicates less than half of the adhesive remained on the tooth; 2 indicates more than half of the adhesive remained on the tooth; and 3 indicates all adhesive remained on the tooth, with a distinct impression of the bracket mesh. The ARI scores were used as a more complex method of defining bond failure site among the enamel, the adhesive, and the bracket base.

Statistical analysis

Descriptive statistics including mean, standard deviation, median, and minimum and maximum values were calculated for the 8 groups. A 2-way analysis of variance (ANOVA) was applied to determine whether significant differences in debond values existed among the various groups. For the post-hoc test, the Scheffé test was used, and the Bonferroni correction was applied.

The chi-square test was used to determine significant differences in the ARI scores among the different groups. Significance for all statistical tests was predetermined at P < 0.05, and for the post-hoc tests according to Bonferroni correction.

All statistical analyses were performed with Stata 7 Program (Stata, College Station, Tex).

RESULTS

Descriptive statistics for shear bond strengths are presented in Table II. The results of the ANOVA indicated significant differences among the groups (P = .000).

When comparing the 2 priming systems, no significant differences were found for the dry enamel groups

 Table II. Descriptive statistics (in MPa) of shear bond strengths of 8 groups tested (each group consisted of 15 specimens)

Primer	Groups	Mean	SD	Min	Median	Max	Scheffé*
СР	1	8.27	1.65	5.15	8.07	13.07	А
	2	3.76	1.37	1.67	3.94	6.10	В
	3	3.64	1.28	1.26	3.81	5.64	В
	4	3.34	1.17	1.83	2.91	5.47	В
HP	1	8.36	2.60	4.05	9.02	11.89	А
	2	4.86	1.15	2.88	4.62	7.35	С
	3	4.69	1.03	2.87	4.68	6.44	С
	4	4.26	0.85	2.71	4.24	5.54	С

* Scheffé grouping: means with same letter are not significantly different.

CP, Conventional primer; HP, hydrophilic primer.



Fig 2. Mean shear bond strengths (MPa) of 2 primers under 4 testing conditions (1, no contamination; 2, blood contamination before priming; 3, blood contamination after priming; 4, blood contamination before and after priming).

(P = .91), whereas, for all blood-contaminated groups (before, after, and before and after priming), the hydrophilic primer showed significantly higher shear bond strengths than the conventional primer (P < .02).

With the conventional primer, group 1 (dry) showed the highest bond strength, which was significantly higher (P = .000) than that of groups 2, 3, and 4 (Fig 2). No statistically significant differences (P > .41) were reported among the remaining groups (2-4). Also, for the groups bonded with the hydrophilic primer, the bond strength of group 1 (dry) was significantly higher than that of all other groups (P = .000). No statistically significant differences (P > .29) were reported among the remaining groups (2-4).

The ARI scores for the 8 groups are listed in Table III. The chi-square test results indicated significant differences among the various groups (P = .000).

No significant differences in the frequency of ARI

score were found between groups bonded with conventional and hydrophilic primers (P = .50).

For groups bonded with the conventional primer, group 1 (dry) had a significantly greater frequency of ARI scores of 3 (P = .000) than the other groups, which showed a greater frequency of ARI scores of 0 and 1. No statistically significant differences were found among the blood-contaminated groups (P > .32).

For groups bonded with the hydrophilic primer, group 1 (dry) had a higher frequency of ARI scores of 2 and was significantly different from groups 3 and 4 (P < .004), which showed a higher frequency of ARI scores of 1. No statistically significant differences were found among the remaining groups.

DISCUSSION

This study showed that noncontaminated enamel surfaces had the highest bond strengths for both conventional and hydrophilic primers. This agrees with previous studies that evaluated the shear the bond strength of conventional¹ and hydrophilic⁹ primers on dry and blood-contaminated enamel.

Moreover, in the present study, the 2 primers showed no significant differences on dry enamel, whereas, when used on blood-contaminated enamel, the hydrophilic primer showed significantly higher shear bond strengths than the conventional primer. Previous studies that compared Transbond XT and MIP under dry conditions have shown conflicting results. Littlewood et al¹⁷ found that the bond strength achieved with the hydrophilic primer was significantly lower than that achieved with the conventional primer, but Webster et al¹⁶ and Grandhi et al¹⁸ reported no significant differences between the 2 primers, as also confirmed in the present study.

Enamel surface contamination can occur at 2 critical times of the bonding procedure: after the tooth surface has been etched and after the primer has been applied. Bonding can be compromised at both times.

In this study, under blood-contaminated conditions, both conventional and hydrophilic primers produced significantly lower bond strength values compared with those achieved under dry conditions, as also reported on saliva-contaminated enamel by Webster et al.¹⁶

For each primer, no significant differences were found among the 3 blood-contaminated groups. Therefore, the time at which the blood contamination occurred during the bonding procedure had no significant influence on the bond strength values. These results agree with those reported on saliva-moistened enamel surfaces.¹⁶

For all blood-contaminated conditions, the hydro-

Primer	Group	Condition	ARI = 0	ARI = 1	ARI = 2	ARI = 3
СР	1	Dry environment	1 (6.7%)	1 (6.7%)	4 (26.7%)	9 (60.0%)
	2	Blood before priming	6 (40.0%)	6 (40.0%)	3 (20.0%)	0 (0.0%)
	3	Blood after priming	4 (26.7%)	8 (53.3%)	3 (20.0%)	0 (0.0%)
	4	Blood before and after priming	8 (53.3%)	4 (26.7%)	2 (13.3%)	1 (6.7%)
HP	1	Dry environment	0 (0.0%)	3 (20.0%)	9 (60.0%)	3 (20.0%)
	2	Blood before priming	3 (20.0%)	6 (40.0%)	5 (33.3%)	1 (6.7%)
	3	Blood after priming	3 (20.0%)	7 (46.7%)	5 (33.3%)	0 (0.0%)
	4	Blood before and after priming	4 (26.7%)	9 (60.0%)	2 (13.3%)	0 (0.0%)

 Table III. Frequency of distribution of adhesive remnant index scores (%)

CP, Conventional primer; HP, hydrophilic primer.

philic primer showed significantly higher shear bond strength values than did the conventional primer.

By applying a layer of Transbond MIP to acidconditioned enamel, in addition to micromechanical retention, a reversible hydrolytic bond mechanism can be established by breaking and reforming of carboxylate salt complexes formed between the ionized carboxyl groups of the methacrylate functionalized-polyalkenoic acid copolymer and residual enamel calcium.¹⁹ This might enhance the bonding onto water-contaminated or salivacontaminated enamel surfaces.¹⁸

Reynolds²⁵ suggested that a minimum bond strength of 6 to 8 MPa was adequate for most clinical orthodontic needs because these values are considered to be able to withstand masticatory and orthodontic forces. In our study, the bond strengths of the 2 priming systems used on dry enamel surfaces were above these limits, but, on blood-contaminated enamel, the minimum requirement was not achieved, independently of the time of blood contamination. Although significantly higher, the bond strength values produced by the hydrophilic primer on blood-contaminated enamel might not be clinically adequate.

According to the present findings and those of previous studies,^{16,18} the conventional primer is ideal for bonding to dry enamel, because it produces shear bond strengths that are significantly higher than those achieved with the same material under blood-contaminated conditions.

Also, the hydrophilic primer performed better on dry enamel than under all other conditions evaluated. These findings are consistent with those of other authors on water-moistened and saliva-moistened enamel.^{1,16,18}

Previous studies showed that bovine and human enamel are similar in their physical properties, composition, and bond strengths.^{26,27} Bovine enamel has been reported to be a reliable substitute for human enamel in bonding studies.²⁶⁻²⁸ Thus, bovine mandibular incisors were used in the present study because they were readily available and inexpensive, and have a close morphologic similarity to human enamel.

The range of ARI scores clearly showed that the conventional primer used under dry conditions had a significantly greater frequency of bond failures at the bracket-adhesive interface (ARI score 3), whereas, when used on blood-contaminated enamel, it debonded more frequently at the enamel-adhesive interface (ARI score 0 and 1), agreeing with the results of Webster et al¹⁶ on saliva-moistened enamel. This finding is probably due to the hydrophobic properties of the primer and the composite.

The hydrophilic primer used under dry conditions had a higher frequency of adhesive failures, with more than half of the adhesive left on the tooth (ARI score 2). On the other hand, blood-contaminated groups had a higher frequency of bond failures at the enamel-adhesive interface (ARI score 1), as also shown by Hobson et al⁹ on blood-contaminated enamel and by Webster et al¹⁶ on saliva-moistened enamel.

CONCLUSIONS

The following conclusions can be made:

1. Noncontaminated enamel surfaces had the highest bond strengths for both conventional and hydrophilic primers; under dry conditions, no significant differences were found between the 2 primers.

2. Under blood-contaminated conditions, both primers produced significantly lower bond strengths. The hydrophilic primer had significantly higher strength values than the conventional primer, but they both had bond strength values that might not be clinically adequate.

3. For each type of primer, no significant differences were reported among the blood-contaminated groups.

4. Both the conventional and the hydrophilic primers showed significant differences in debond failure sites, depending on the various enamel surface conditions. 5. Based on the results of this study, hydrophilic primers can be successfully used for bonding adhesiveprecoated brackets under dry conditions; however, blood contamination of the enamel surface during the bonding procedure significantly lowers their bond strength values.

We thank 3M/Unitek for providing the materials tested in this study.

REFERENCES

- 1. Xie J, Powers JM, McGuckin RS. In vitro bond strength of two adhesives to enamel and dentin under normal and contaminated conditions. Dent Mater 1993;9:295-9.
- Plasmans PJ, Creugers NH, Hermsen RJ, Vrijhoef MM. The influence of absolute humidity on shear bond adhesion. J Dent 1996;24:425-8.
- Sheen DH, Wang WN, Tarng TH. Bond strength of younger and older permanent teeth with various etching times. Angle Orthod 1993;63:225-30.
- Ehudin DZ, Thompson VP. Tensile bond strength of dental adhesives bonded to simulated caries-exposed dentin. J Prosthet Dent 1994;71:165-73.
- O'Brien JA, Retief DH, Bradley EL, Denys FR. Effects of saliva contamination and phosphoric acid composition on bond strength. Dent Mater 1987;3:296-302.
- Krejci I, Lutz F, Perisic U. The effects of the processing technic on dentinal adhesion. Schweiz Monatsschr Zahnmed 1992;102: 924-9.
- Johnson ME, Burgess JO, Hermesch CB, Buikema DJ. Saliva contamination of dentin bonding agents. Oper Dent 1994;19: 205-10.
- Powers JM, Finger WJ, Xie J. Bonding of composite resin to contamined human enamel and dentin. J Prosthodont 1995;4:28-32.
- 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.
- Wisth PJ, Nordenval K, Boe OE. Periodontal status of orthodontically treated impacted maxillary canines. Angle Orthod 1976; 46:69-76.
- Kohavi D, Becker A, Zilberman Y. Surgical exposure, orthodontic movement, and final tooth position as factors in periodontal breakdown of treated palatally impacted canines. Am J Orthod 1984;85:72-7.
- 12. Zachrisson BJ. A posttreatment evaluation of direct bonding in orthodontics. Am J Orthod 1977;71:173-89.

- Gwinnett AJ. Bonding of restorative resins to enamel. Int Dent J 1988;38:91-6.
- 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.
- Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. J Am Dent Assoc 1985;110:329-32.
- 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.
- Littlewood SJ, Mitchell L, Greenwood DC, Bubb NL, Wood DJ. Investigation of a hydrophilic primer for orthodontic bonding: an in vitro study. J Orthod 2000;27:181-6.
- Grandhi RK, Combe EC, Speidel TM. Shear bond strength of stainless steel orthodontic brackets with a moisture-insensitive primer. Am J Orthod Dentofacial Orthop 2001;119:251-5.
- Eliades T, Katsavrias E, Eliades G. Moisture-insensitive adhesives: reactivity with water and bond strength to wet and saliva-contaminated enamel. Eur J Orthod 2002;24:35-42.
- Schaneveldt S, Foley TF. Bond strength comparison of moistureinsensitive primers. Am J Orthod Dentofacial Orthop 2002;122: 267-73.
- Jobalia SB, Valente RM, de Rijk WG, BeGole EA, Evans CA. Bond strength of visibile light-cured glass ionomer orthodontic cement. Am J Orthod Dentofacial Orthop 1997;112:205-8.
- Millett DT, Cattanach D, McFadzean R, Pattison J, McColl J. Laboratory evaluation of a compomer and a resin-modified glass ionomer cement for orthodontic bonding. Angle Orthod 1999; 69:58-63.
- 23. Sfondrini MF, Cacciafesta V, Pistorio A, Sfondrini G. Effects of conventional and high-intensity light-curing on enamel shear bond strength of composite resin and resin-modified glassionomer. Am J Orthod Dentofacial Orthop 2001;119:30-5.
- Årtun J, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. Am J Orthod 1984;85:333-40.
- Reynolds IR. A review of direct orthodontic bonding. Br J Orthod 1975;2:171-8.
- Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. J Dent Res 1983;62:1076-81.
- Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop 1998;114:514-9.
- Barkmeier WW, Erickson RL. Shear bond strength of composite to enamel and dentin using Scotchbond Multi-Purpose. Am J Dent 1994;7:175-9.