Influence of decontamination procedures on shear forces after contamination with blood or saliva

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Introduction: Despite rapid development in adhesive technology, contamination of bonding surfaces remains a major problem. The aims of this study were to evaluate the influence of contamination on bond strength and to investigate possible decontamination procedures. Methods: Four bonding systems were evaluated for their shear bond strengths under 5 bonding situations: control (without contamination and decontamination); contamination with blood; contamination with saliva; decontamination with water and air, and repriming after blood contamination; and decontamination with water and air, and repriming after saliva contamination. The 25 specimens of each group consisted of composite blocks bonded to bovine teeth. Shear forces were measured with a testing machine after thermocycling. Results: The 3 composite primers showed similar behavior. With the exception of Transbond SEP (3M Unitek, Monrovia, Calif) with saliva contamination, all contaminated samples showed greatly reduced shear forces. The control and decontaminated groups showed shear forces about 20 MPa. The resin-modified glass ionomer, however, did not reach clinically sufficient bond strengths in either setup. Conclusions: Decontamination with water and air and repriming is sufficient after contamination with blood or saliva. Etching again is not necessary. The bond strength of Transbond SEP was not significantly altered by saliva contamination and can be recommended for conventional bonding procedures. (Am J Orthod Dentofacial Orthop 2010;138:435-41)

Since its introduction in 1955 by Buonocore, adhesive techniques have had tremendous success in diverse fields of dentistry. However, it is still difficult to maintain perfect bonding conditions for these techniques in the oral cavity. In particular, contamination with blood or saliva is frequent.

To overcome these problems, adhesives more tolerant to humidity have been developed in the last decade. One modification to achieve this is to add acetone or ethanol to the primer as a solvent. Both substances can displace water and should therefore be less sensitive to contamination. Clinically, the necessity for a dry environment still remains. Another class of substances that are known to be more hydrophilic than conventional composites are resin-modified glass ionomers. Ionic linkage between the hydroxyapatite and the carboxylate of the polycarboxylic acid allows for application to unetched enamel. The additional application of a conventional etching agent, however, enhances bonding strength significantly.

Another possibility to minimize contamination is to reduce the clinical steps of the bonding procedure and thus eliminate sources of contamination, as has been done with self-etching primers. Acid monomers are used in self-etching primers. They dissolve the enamel surface and, by doing so, release calcium ions. This causes neutralization of the acid monomers, and thus the progression of etching is stopped, and equal penetration of etching and bonding is guaranteed. In addition, higher tolerance against contamination with water or saliva has been observed.

In addition to the maintenance of a dry environment, the development of humidity-tolerant adhesives, or the reduction of opportunities for contamination, a fourth approach can be considered: decontamination. Little is known about successful decontamination procedures after blood or saliva contamination of a previously etched or even an already bonded tooth surface. Because procedures involving renewed etching of tooth surfaces cause further enamel loss, we focused on the clinical possibilities of decontamination by water and air and repriming without renewed etching.
Many studies have evaluated the influence of contamination on shear bond strengths.\cite{3,6,7,9,10,18,22,23} The most critical times for contamination are immediately before and after the primer has been applied to the tooth.\cite{7,23,24} Contamination before priming would inevitably cause the formation of a smear layer.\cite{5} This layer consisting mainly of proteins covers the etched surface within seconds and thus inhibits the penetration of the porous surface by the priming agents.\cite{4,5,13,25} Contamination can also take place after priming. Fewer hydrophobic adhesive bonds will cause reduced adhesive strength.\cite{5,12,25} Both situations result in inferior adhesive strength\cite{7,23,26} and might lead to early detachment of the brackets or retainers.\cite{7,18,26}

Our aim was to evaluate which substance is least affected by contamination with saliva or blood after priming, and whether decontamination without renewed etching can reestablish acceptable bond strengths.

### MATERIAL AND METHODS

Four bonding systems were evaluated for their adhesive properties in different bonding situations (Table I). System 1 comprised etching with 35% phosphoric acid, Transbond XT primer and Transbond XT adhesive (3M Unitek, Monrovia, Calif). System 2 comprised the same etching procedure and adhesive, but the moisture-insensitive primer Transbond MIP (3M Unitek) was used. System 3 consisted of the self-etching primer Transbond Plus SEP (3M Unitek) combined with Transbond XT adhesive. In system 4, the resin-modified glass ionomer Fuji Ortho LC (GC America, Alsip, Ill) was used with 10% polyacrylic acid Ortho Conditioner (GC America).

Each bonding system was divided into 5 groups according to the contamination and decontamination procedures: (1) ideal bonding condition, (2) contamination with blood, (3) contamination with saliva, (4) contamination with blood and decontamination with water and air followed by repriming without etching, and (5) contamination with saliva and decontamination with water and air followed by repriming without etching.

Each group consisted of 25 specimens, for a total of 500 shear force settings. Freshly extracted bovine deciduous incisors were used as the enamel substrate. The teeth were controlled for macroscopic deficiencies in the enamel structure. Only teeth with visually intact enamel surfaces were selected for testing. For hygienic reasons, the teeth were cleaned of soft tissue, and the pulp was extirpated. Thereafter, they were stored in physiologic sodium chloride for a week before shear bond strength testing.

The buccal surfaces of all teeth were pumiced. For systems 1 and 2 (Transbond XT and MIP primer), the teeth were etched for 15 seconds and rinsed with water for 10 seconds, drying with air. Whereas the specimens of system 1 were dried with air for 20 seconds, tooth surfaces of system 2 were kept moistened. Both primers were applied for 10 seconds and dispersed with an air flow for 5 seconds.

In system 3 (Transbond SEP Plus), no etching was done. Transbond SEP was applied for 5 seconds and dispersed with an air flow for 2 seconds.

In system 4, GC Ortho...
Conditioner was used for an etching period of 20 seconds, followed by thorough rinsing for 30 seconds. A slight humidity was preserved by gentle drying with air.

Whereas group 1 was the uncontaminated control group, groups 2 and 3 were contaminated with blood and saliva, respectively, for 10 seconds before application of the adhesive. Both blood and saliva were collected from the examiner (M.E.). Groups 4 and 5 were contaminated in the same manner as groups 2 and 3, but they were decontaminated with water for 10 seconds. Thereafter, these groups were reprimed according to the normal protocol. In system 4 (Fuji Ortho LC), the contamination and decontamination procedures were also performed before application of the adhesive. However, because there was no priming agent, the contamination was applied to the etched enamel.

Composite blocks with a bonding surface of 12.6 mm² and a height of 5 mm were used for shear bond testing. For this, highly precise cylinders were constructed from stainless steel by a computerized numerical control mill (Picomax 60-M/HSC, Fehlmann AG, Seon, Switzerland). A negative impression was taken by using low viscosity silicone (Finosil, Fino, Schweinfurt, Germany). The negative cylinders formed by the impression were filled with a flowable composite (Grandioflow, VOCO, Cuxhaven, Germany) and light cured (Bluephase, Ivoclar Vivadent, Lichetenstein).

After bonding the composite blocks to the teeth, the probes were subjected to 1000 temperature cycles between 5°C and 55°C within 50 hours (Circulator C-85, Techne, Stone, UK; Julabo UC and 5B, Julabo Laborotechnik, Seelbach, Germany).

To obtain a bonding interface parallel to the shear force vector, a positioning gauge was used. The teeth were embedded in a polymethacrylate socket (Technovit, Heraeus Kulzer, Wehrheim, Germany) and tested for maximum shear force with a universal testing machine (model 4444, Instron, Wilmington, Del). In addition, the adhesive remnant index (ARI) scores were evaluated for all samples, by estimating the amount of bonding material remaining on the 2 surfaces under 3.5-fold magnification. A score of 0 was used for samples with no adhesive left on the enamel, 1 for less than 50% on the enamel, 2 for more than 50% on the enamel, and 3 when all adhesive remained on the tooth.

Statistical analysis

SAS software (version 9.1, SAS, Cary, NC) was used for statistical analysis. Means, medians, minimum and maximum values, and standard deviations were calculated. The nonparametric Kruskal-Wallis test was used to rank the results of the different groups. Significance was set at $P < 0.05$ and calculated with the Mann-Whitney U test. Samples that could not be measured because of spontaneous detachment during thermocycling were evaluated as 0 bond strength.

RESULTS

Mean shear force values were about 20 MPa for the control and the decontamination groups of Transbond XT, MIP, and SEP as well as the saliva contamination group of Transbond SEP. All other shear fractures occurred at about 5 MPa (Fig, Table II). The shear values showed a direct correlation to the ARI scores, achieving high ARI scores for groups with high bond strengths (Table II). This resulted in a correlation factor of $r = 0.87$ according to the Spearman nonparametric calculation. Fuji Ortho LC showed significantly lower values for the control group and both decontamination
groups, whereas the 3 composite systems showed no significant differences between the control and decontamination groups or between the different materials (Table III). For the contamination groups, Transbond XT showed small but significantly higher bond strengths than its competitors, and Transbond SEP had highly significant advantages in bond strength with saliva contamination (Table IV).

Many samples displayed spontaneous detachment during the thermocycles. These detachments occurred in the contamination groups of the composite bonding systems and in all groups of the resin-modified glass ionomer (Table II). Only a few enamel fractures were recorded for the bonding systems except for Transbond Plus SEP, which showed enamel fractures in 16.8% of all samples (Table II).

### DISCUSSION

Adhesive techniques such as bracket bonding are extremely sensitive to contamination. With conventional adhesive techniques, clinically adequate bonding strengths can be obtained only in a dry environment. Contamination of bonding surfaces, however, is a common problem in orthodontics, especially in young children and disabled patients, and for lingual techniques in the mandible and generally for the posterior dentition. Therefore, the aim of this study was to evaluate the influence of contamination with blood or saliva as well as decontamination with water and air on bonding strengths to the tooth surfaces.

In contrast, of the studies looking at bonding strength in an ideal environment, only a few have attempted to clarify the value of possible decontamination procedures. In general, since all the studies looked at different variables, such as thermocycling, bonding substrate, shear mechanism, or decontamination procedure, a direct comparison with our results is impossible. A few of these variables—bonding substrate and contamination or decontamination procedures—can be compared. Application of some form of stress, either thermal or mechanical, to the bonding site before shear force testing to simulate the microstresses to which bonding sites are exposed during the average 2 years of orthodontic treatment might be advisable. Several articles showed that thermocycling reduced the bonding strength significantly. Another critical factor is the mode of shear force testing. To test bonding strength between the enamel and the substrate, it is important to minimize fracture sites that are not located at this interface. Whereas brackets bonded to teeth are useful to evaluate the bonding strength of the whole tooth-adhesive-bracket structure, they are inappropriate if only the tooth-adhesive interface is evaluated. In particular, metal brackets have lower bond strengths to composite than enamel.

<table>
<thead>
<tr>
<th>System</th>
<th>Group</th>
<th>Mean (Mpa)</th>
<th>SD (Mpa)</th>
<th>ARI score</th>
<th>Enamel fracture (n)</th>
<th>Early detachment (%)</th>
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<td>Transbond XT</td>
<td>1 (control)</td>
<td>25.06</td>
<td>10.81</td>
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<td>—</td>
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<td></td>
<td>2 (blood)</td>
<td>4.88</td>
<td>4.82</td>
<td>0.44</td>
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<td>12</td>
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<tr>
<td></td>
<td>3 (saliva)</td>
<td>4.75</td>
<td>5.88</td>
<td>0.56</td>
<td>1</td>
<td>24</td>
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<td></td>
<td>4 (blood, decon)</td>
<td>21.37</td>
<td>9.81</td>
<td>2.12</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5 (saliva, decon)</td>
<td>20.09</td>
<td>11.13</td>
<td>1.72</td>
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<td>Transbond MIP</td>
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<td>20.53</td>
<td>10.79</td>
<td>1.96</td>
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<td></td>
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<td>1.51</td>
<td>0.16</td>
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<td>11.04</td>
<td>1.68</td>
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<td>10.53</td>
<td>2.68</td>
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<td>3.29</td>
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<td>2.81</td>
<td>0.2</td>
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Decon, Decontaminated.
shear force testing, we suggest composite blocks. It is also much easier to align a geometric form, such as a cylinder, parallel to the shear axis than a bracket; this allows for better standardization of the shear mechanism.

The most common contaminants of enamel during bonding procedures are saliva and blood. Whereas saliva occurs in all bonding situations, blood is mainly a problem when rebonding brackets if there is gingivitis. Saliva consists mostly of water (99%), polysaccharides, proteins, and enzymes. The mechanism of contamination occurs in different ways. It seems that, within seconds, an organic smear layer is formed, covering the etched porous surface. In addition to the mechanical adherence of the bonding agent, chemical binding between calcium and phosphate ions has also been described.

Our study showed differences in the bonding characteristics of the 4 systems on contaminated surfaces. The hydrophobic properties of Transbond XT primer repelled saliva. However, the effect was not sufficiently strong, and bond strengths were significantly lower than those in the control group. These results agree with other studies. The moisture-insensitive Transbond MIP primer showed similar behavior. It seems that ethanol, which is responsible for humidity tolerance, can enhance the adhesive strength when combined with minimal humidity such as sulcus fluid or moist breath. Saliva contamination, however, covers the surface with not only considerable amounts of moisture, but also substantial organic material. In this study, the binding force was reduced by approximately 80%; this correlates with previous investigations. A different behavior was observed with the self-etching primer Transbond SEP. The study showed that binding forces are almost unaffected by saliva contamination; this agrees with earlier investigations. Denturation and degradation of saliva proteins by the etching components are probably responsible for this behavior. Fuji Ortho LC had the best results in a humid environment whether by saliva contamination or water. However, the results were not significantly different from bonding in a dry environment; this was contrary to some studies. The shear forces were in a clinically unacceptable range, below the 6 to 8 MPa recommended by Reynolds. This difference in comparison with previous studies might be explained by the use of thermocycling in this investigation. Many specimens (8%-88%) showed spontaneous detachment after thermocycling. Glass ionomers seem to be more susceptible to external stress factors than composites.

In contrast to the contamination with saliva tolerated by Transbond SEP and Fuji Ortho LC, contamination with blood led to uniformly and highly decreased bonding forces. Our study supports the results of previous investigations on bonding strength after blood contamination. It seems that high amounts of organic substances impede the binding between primer and adhesive. Protection of the bonding sites...
from blood contamination is therefore essential, especially for surgical exposure of retained teeth.

The second aim of this study was to evaluate a possible decontamination procedure when contamination has already occurred. Certainly, complete repetition of all bonding steps would again lead to optimal adhesion. However, repeated etching would also cause further enamel loss. A decontamination procedure that does not involve repeated etching would therefore be preferable. We found that cleaning a contaminated surface with water and air was sufficient to obtain adequate bonding forces. For all composite groups, bond strength after decontamination without repeated etching was only insignificantly lower than for the control group without contamination. Adhesion was even enhanced after decontamination for Fuji Ortho LC but still remained below the 6 to 8 Mpa for successful bonding. These results support our clinical experience that, after blood or saliva contamination, renewed etching is not necessary. This observation contrasts with previous studies that found that bonding forces remain significantly reduced after decontamination but agrees with the investigations of Eiriksson et al., Ghavam, and Sayinsu et al. A direct comparison, however, is impossible because of different methodologies. Webster et al. compared a control group with a contamination group (without repriming) and a decontamination group (repriming only). They found significantly reduced bonding forces in both situations. The decontamination in the investigation by Zeppieri et al. also consisted of repriming without previous rinsing with water. Only Transbond Plus SEP achieved successful bonding forces in the decontamination groups. In the studies by Eiriksson et al., all self-etching primers achieved successful bonding forces after decontamination with water, air, and repriming. Ghavam found that decontamination with water and air followed by repriming was successful for both previously light cured or uncured priming. Similar results were found by Sayinsu et al. Overall, modern adhesives seem to be able to successfully bond to decontaminated surfaces. On the other hand, contamination might cause alterations in enamel color by degradation of salivary or blood proteins trapped in the bonding. However, this could not be addressed in this study.

CONCLUSIONS

Our investigation showed that decontamination consisting of thorough rinsing with water, drying with air, and repriming can be used successfully on surfaces contaminated with blood or saliva after priming. Contamination with blood without decontamination, however, resulted in strongly reduced bond strengths for all tested adhesives. Interestingly, Transbond SEP was not affected by saliva contamination, whereas the other groups all showed strongly reduced bond strengths. Clinical management of contaminated bonding surfaces can be recommended as follows: renewed etching is not necessary. Simple decontamination with water, air, and repriming gives sufficient bond strength for all tested adhesives. For Transbond SEP and saliva contamination, repriming caused only adequate adhesion.

We thank the manufacturers that supplied the materials for this study.

REFERENCES

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