Effects of blood contamination on resin-resin bond strength

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Resin composite; Blood contamination; Adhesion; Dental materials; Dentin bonding agent

Summary

Objective. Incremental placement and curing of resin composites has been recommended. However, this requires longer operating time, and therefore, increased risk of contamination. The purpose of this study was to evaluate the effects of blood contamination on microtensile bond strengths ($\mu$TBS) between resin interfaces and to determine the best decontamination method to re-establish the original resin–resin bond strength.

Materials. The top surfaces of 64, 4-mm composite blocks (Z-250, Renew, APX, Pertac II) were untreated as the control, or were treated as follows: blood applied and dried on the surface (Treatment 1), blood applied, rinsed, dried (Treatment 2), blood applied, rinsed, and an adhesive applied (Single Bond, One-Step, Clearfil SE, Prompt L-Pop) (Treatment 3). Fresh composite was applied and light-cured in 2-mm increments. After 24 h storage in water, the specimens were sectioned into 0.7-mm thick slabs, trimmed to a cross-sectional area of 1 mm\textsuperscript{2}, and loaded to failure at a crosshead speed of 1 mm/min using an Instron universal testing machine. Data were analyzed using two-way ANOVA and Fisher's PLSD test ($p < 0.05$).

Results. Control values ranged from 45.1 MPa for Pertac II to 71.5 MPa for APX. Untreated blood contamination resulted in resin–resin bond strengths of only 1.0–13.1 MPa. Rinsing raised bond strengths to over 40 MPa for each material. Use of an adhesive further increased bond strengths except for Pertac II.

Significance. Rinsing blood from contaminated surfaces increases the resin–resin bond strength significantly and the application of an appropriate adhesive increases the bond strength to control levels.

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Introduction

Achieving good moisture control is a common problem encountered in restorative dentistry, especially when rubber dam isolation is not feasible. The popularity of dental composites has increased rapidly in the last decade and drawn attention to the importance of moisture and contamination control, because according to basic concepts of adhesion, the closer the contact between the adhesive and adherent, the stronger is their junction.\textsuperscript{1} Good bonding to the tooth...
surface is necessary for retention and to prevent microleakage around margins of restorations. In addition, clinicians are encouraged to place resin composite restorations in increments to ensure bonding to the surrounding tooth structure and complete polymerization of large restorations for optimal physical properties. Gap formation between bonded restoration and preparation walls can be caused by stress formation from polymerization shrinkage.

The longer time required for placement and polymerization of composite increments at margins below gingival tissue makes contamination control more difficult. A few studies have been executed on dentin bonding agents involving saliva and blood contaminated tooth surfaces; however, there is a lack of information on the effect of contamination between resin increments.

One-bottle dental adhesives combine the primer and adhesive components of multi-step bonding systems in a single solution. Acetone or ethanol solvents in the one-bottle adhesives remove residual moisture and enhance resin wetting of the substrate. More recently, two-step self-etching primer systems, which combine the etchant and primer in one bottle and the adhesive in a separate bottle, as well as self-etching adhesives that combine etch, primer, and adhesive in a single solution, have become available. The role of these various new dentin bonding systems in decontaminating resin increments that are contaminated with blood during placement of the composite restoration needs to be evaluated.

The purpose of this study therefore was to evaluate the effect of blood contamination on microtensile bond strength (μTBS) between resin increments and to determine the best decontamination method to re-establish the original resin-resin bond strength. The hypothesis tested was that blood contamination would reduce resin-resin microtensile bond strength, but that some adhesives could reverse this effect.

### Materials and methods

Four commercially available resin composites and adhesives were used. The adhesives, compositions, batch numbers and manufacturers are listed in Table 1. Single Bond (SB), an ethanol-based adhesive, and One-Step (OS), an acetone-based adhesive were used as the one-bottle adhesives. Clearfil SE Bond (SE) was used as the two-step self-etching primer system, and Prompt L-Pop (LP) was the all-in-one self-etching adhesive. Each adhesive was used with the respective Bis-GMA based hybrid resin composite from the same manufacturer. SB was used with zirconia-filled Z-250, OS with barium glass-filled Renew, SE with barium glass-filled Clearfil APX and Prompt LP with quartz-filled Pertac II.

The adhesives were applied and cured according to the manufacturer’s instructions (Table 1). A transparent vinyl cylinder 4 mm high x 10 mm wide was filled with resin composite. A spatula was used to remove the excess in order to produce a relatively flat surface, and the composite was light-cured from the top and bottom surfaces of the cylinder for 60 s each to ensure complete polymerization of the composite, using a visible light source.

### Table 1 Composition of the bonding agents and the composites.

<table>
<thead>
<tr>
<th>Adhesives</th>
<th>Composition</th>
<th>Batch no.</th>
<th>Bonding instruction++</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single bond</td>
<td>Bis-GMA, HEMA, dimethacrylates, polyalkenoic copolymer, ethanol, water, photoinitiator</td>
<td>9CL</td>
<td>c(2x),b,d(10 s)</td>
<td>3M ESPE St. Paul, MN</td>
</tr>
<tr>
<td>One step Clearfil SE bond primer</td>
<td>Bis-GMA, BPDM, HEMA, acetone</td>
<td>9900010095</td>
<td>c(2x),d(10 s)</td>
<td>BISCO Inc. Schaumburg, IL</td>
</tr>
<tr>
<td>Clearfil SE bond adhesive</td>
<td>MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, n,n Diethanol-p-Toluidine, camphoroquinone(CQ), water</td>
<td>61119</td>
<td>c(15 s),b,d(10 s)</td>
<td>Kuraray Co., Ltd, Tokyo, Japan</td>
</tr>
<tr>
<td>Prompt L-Pop</td>
<td>Water, methacrylated phosphoric acid esters, fluoride complex w/ zinc, parabenes</td>
<td>61414</td>
<td>c(15 s),b,d(10 s)</td>
<td>3M ESPE St. Paul, MN</td>
</tr>
</tbody>
</table>

Abbreviations. Bis-GMA: bisphenol-A-glycidylmethacrylate, HEMA: Hydroxyethyl methacrylate, BPDM: bisphenyl dimethacrylate, MDP: 10-methacyrloxy methacrylate; ++ Bonding procedures as executed in treatments 4 and 5: (a) apply primer, (b) mild air flow, (c) apply adhesive (d) light cured.
The composite cylinders were randomly divided into four groups according to the different surface contamination/treatments. Four cylinders were fabricated per group for each material, for a total of 64 composite cylinders. Blood was collected from a single individual (a needle-prick to alcohol wiped forefinger) and was collected at the time of experiment. It has been shown that freshly drawn capillary blood is more suitable in laboratory experiments involving blood contamination than heparinized blood.17 The groups were treated as follows:

Control group. The control group was not contaminated with blood or treated with adhesive; instead, two layers of composite were added to the original cured composite.

Treatment 1. Blood was applied to the specimens using a microbrush (Kerr Corporation, Orange, CA) and dried carefully with oil-free compressed air for 20 s from a distance of 10 cm. Care was taken to maintain a layer of dry blood on top of the samples.

Treatment 2. Blood was applied and dried as previously described. Air/water spray was then used to rinse off the contamination for 20 s followed by air-drying for 20 s.

Treatment 3. Blood application and rinsing were done as in treatment 2, and an adhesive was applied to the surface and light-cured as recommended (Table 1).

After surface treatment, the periphery of the specimen was marked with permanent ink to ensure that the interface could be detected when cutting the samples. Each treated surface was ‘restored’ with two 2-mm increments of resin composite, with each being light-cured using the Optilux 501 for 40 s. The bonded assemblies were stored for 24 h in distilled water at 37 °C.

A diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) was used to cut the specimens into 0.7-mm² thick slabs. Each slab was trimmed into an hourglass shape using a fine diamond on a water-irrigated high-speed handpiece to a cross-sectional area of 1.0 ± 0.2 mm².18 A digital micrometer was used to measure the thickness and width of the bonded area of each specimen. Three specimens were sectioned from each cylinder, for a total of 192 specimens (12 per material per group). The specimens were attached with cyanoacrylate glue (Zapit, DVA, Corona, CA, USA) to a Bencor Multi-T testing apparatus (Danville Engineering, Danville, CA, USA) mounted in an Instron universal testing machine (model 4411, Instron Corporation, Canton, MA, USA) at a crosshead speed of 1 mm/min. Maximum load at fracture divided by the cross-sectional surface area of the bonded surface was used to calculate the microtensile bond strength. If spontaneous interfacial debonding occurred while the specimens were being mounted or sectioned, the bond strength was recorded as 0 MPa. The data were analyzed using two-way and one-way ANOVA with Fisher’s PLSD (p < 0.05).

After debonding, the specimens were fixed in 10% neutral buffered formalin for at least 8 h19 to decontaminate the specimens. To evaluate the fracture pattern, three representative specimens from each group were chosen randomly. Both debonded sides of the fractured specimens were trimmed, placed on stubs, desiccated in ambient conditions, gold sputter-coated (5100 sputter-coater, Polaron Equipment Ltd, Watford, England), and examined by two investigators using a JSM 6300 scanning electron microscope (SEM, JEOL USA Inc., Peabody, MA, USA). The debonded sides were evaluated for mode of fracture and difference in texture from control groups.

Results

Microtensile bond strengths are summarized in Fig. 1. Two-way ANOVA showed that the factors, ‘surface treatments’ and ‘materials’, and their interaction were significant (p < 0.0001). One-way ANOVA and Fisher’s PLSD test revealed that drying the blood on top of the specimens (treatment 1) resulted in significant reductions in resin-resin bond strengths for all materials (p < 0.0001), with values in the range of only 1.6 (Z-250) to 18.3% (APX) of the control values Fig. 1. Rinsing blood with air/water spray (treatment 2) did not
return bond strengths to the control level for Z-250 (p < 0.0001), Renew (p = 0.0055) and APX (p = 0.0005), but did for Pertac II (p = 0.4555). The addition of an adhesive increased bond strength for Z-250/SB, but not significantly (p = 0.0232) and not to the level of the control group (p = 0.0129). Adhesive application raised the bond strength significantly for Renew/OS and APX/SE and to
ments (Figs. 2a,b and 3a,b). Treatment 2 specimens revealed a smooth surface on the specimens in treatment 1 when compared to the other treatments (Figs. 2a,b and 3a,b). Treatment 2 specimens had a few craters or blisters indicating water or blood might still have been trapped in the composite buildup (Figs. 2c and 3c). A mixture of cohesive failure in composite and adhesive in both base and first increment was observed under the SEM for treatment 3.

Discussion

These four different bonding agents/composites were chosen to represent different types of bonding agents on the market; ethanol and acetone based one-bottle adhesives, self-etching primer system and self-etching adhesive system all used with hybrid resin composite.

To simulate in vivo manipulation of composite surfaces prior to application of a second increment, the resin composite cylinders were fabricated with a composite spatula, thus making it impossible to reproduce perfectly flat composite surfaces for tensile testing. Although ideally the specimens should exhibit flat surfaces to avoid encountering complex stresses during testing, the spatula technique was chosen, because it more closely paralleled the clinical situation of composite contamination and maintained the oxygen-inhibited layer. Because the surface area tested in the μTBS test is much less than the conventional tensile and shear bond strength tests, possible irregularities created with the spatula compared to the trimmed specimens that have been polished with 600-grit discs may not play as much difference as they would have in the conventional tests.

In this study, blood dried on the surface caused a catastrophic decrease in bond strength between resin increments, which is in agreement with published results. Scanning electron microscopic observation revealed a smooth surface on the specimens when compared to the other treatments (Figs. 2a,b and 3a,b) and probably resulted in reduced bond strengths due to lack of interaction of the increment with the contaminated surface.

Rinsing blood before adding new layers of composite raised the resin–resin bond strength to 67% of control values for Z-250, 72% for APX, 76% for Renew and to the control values for Pertac II. SEM observation showed craters indicating that water or blood may still be left on the surface following rinsing and drying (Figs. 2c and 3c). Kaneshima et al. 2000 applied and rinsed blood contamination of acid-etched dentin. They found reduced bond strength unless the most superficial layer (exposed collagen) was removed from the surface before or after contamination with hypochlorite solution. They found no morphologic differences under SEM and concluded that large blood corpuscle elements could be completely rinsed away but reaction between the exposed collagen meshwork and the blood protein components could inhibit primer infiltration into dentin. In this study, no collagen was present but treatment 2 still showed lower μTBS values than control groups for all but one material. A possible explanation might be that either the blood protein components were not rinsed away completely, the water not removed completely or that rinsing caused lowered surface tension of the cured composite and, therefore, caused less wetting of the composite surface with the next composite layer.

The addition of an adhesive significantly increased the resin–resin bond strength of all materials except for the Pertac II/LP group. This may have been caused by moisture or blood protein components trapped on the composite surface interfering with bonding ability or by failure of the bonding agent to form a uniform surface coating. It has been reported that Prompt LP might not successfully be polymerized in thin layers, which could account for the relatively low results when compared to the other treatments/materials. Additional coats of the adhesive system might solve this problem. The solvent in Prompt LP is simply water and therefore more careful drying is necessary to ensure complete removal of the solvent. It has also been reported that the acidity of LP (pH 1.5) may interfere with curing of the resin composite if curing is delayed.

The μTBS values for Renew/OS and APX/SE in Group 3 increased significantly from Group 2 and to a non-significant difference from control groups. The increase for Z-250/SB was not significant (p = 0.1315). The μTBS on the other hand decreased significantly for PertacII/LP (p = 0.0048). Kaneshima contaminated primed dentin with blood, rinsed it off and found statistically lower bond strengths when resin composite was added without a new primer layer of but a non-significant difference from control when the primer was re-applied. In a study by Xie et al., plasma lowered bond strengths by 33–70% for both enamel and dentin but re-etching restored the bond strengths, which confirmed results from protein-contaminated dentin surfaces. This is confirmed
in our study where we found an increase in bond strength for three of the materials tested when bonding agent was used.

OS is an acetone-based primer/adhesive, and when it comes in contact with a moist dentin substrate, the boiling point of acetone is increased and that of water reduced. This causes water and acetone to evaporate, allowing the resin to remain. In this study, OS even though applied to thoroughly dried resin-contaminated surfaces, showed values statistically similar to control values. However, values for ethanol/water-based SB and water-based LP were statistically lower than for their control values. SE also is an ethanol-based primer and adhesive, and ethanol has water removing capabilities similar to acetone. The two adhesives that did not reach the control values contain water as the solvent. It is a co-solvent in SB and the solvent in LP. Because of the lower volatility of the water solvent it can be speculated that SB and LP may more subject to remaining water or blood on the composite surface. It has been reported that when water-based Syntac SC comes into contact with a moist surface, it might become diluted and its bonding efficacy reduced. Water also has been shown to prevent the HEMA molecules from saturating the collagen, because water will not evaporate as easily and completely as does acetone in acetone-based adhesives. SB also contains HEMA and polyalkenoic acid which have been shown to facilitate bonding to wet dentin. The composite surfaces were rinsed and dried thoroughly for 20 s, and no remaining moisture was observed on the surfaces. The statistical differences could be a mere application error, since careful drying is more difficult with water-based bonding agents.

When contamination occurs, both resin and tooth surfaces are usually involved. If tooth surfaces are involved decreased bond strength may lead to leakage and early caries formation. Early bond failures between resin increments due to insufficient bond strength result in stained lines and become unsatisfactory to the patient. This research shows that early methods reported for saliva and blood contamination on enamel and/or dentin work equally well for resin increments and may be used for the benefit of both.

The published studies that deal with the effects of blood contamination on adhesive restorations are limited and comparisons between them are difficult because bonding systems, time points of contamination, type of substrate, type of blood (fresh or anticoagulated) and outcome variable differ between studies. A few researchers used freshly drawn blood, others anticoagulated blood or plasma. Dietrich et al. 2002 found significantly higher percentages of marginal openings after contamination with fresh capillary blood compared to anticoagulated blood. Blood contamination is usually accompanied mixed with saliva/gingival fluid but researchers usually report saliva and blood contamination separately for increased control and comparison.

Conclusion

Blood contamination significantly reduced the bond strengths between resin composite increments regardless of the materials evaluated. However, rinsing with water restored the bond strength significantly for all materials. Within the limitations of this study, it was concluded that rinsing and application of a dentin adhesive appears to be necessary whenever blood contamination exists on a resin surface to ensure better interfacial bonding of the next increment. Nevertheless, the most important factor for ensuring good resin–resin bonding is to avoid blood contamination with proper isolation.

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