Effects of saliva contamination on resin-resin bond strength

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Summary
Objective. The purpose of this study is to evaluate the effects of saliva contamination on microtensile bond strength (μTBS) between resin interfaces and to determine which decontamination methods best re-established the original resin-resin bond strength.

Materials and methods. Ninety-six light-cured resin composite cylinders of Z-250, Renew, Clearfil APX, and Pertac II were randomly divided into six groups. For each material, one group of specimens was not contaminated, serving as the control. For the other specimens, the top surface of each block was treated with saliva that was slowly dried (Treatment 1); dried forcefully (Treatment 2); slowly dried, rinsed, and dried (Treatment 3); slowly dried, rinsed, and bonded with Single Bond, One-Step, Clearfil SE Bond, or Prompt L-Pop (Treatment 4); or slowly dried, but not rinsed, and bonded using the same adhesives (Treatment 5). Two 2-mm increments of resin composite were applied and light-cured. After 24 h, the assemblies were trimmed for microtensile bond testing and were loaded to failure at 1 mm/min. Data were analyzed using two-way and one-way ANOVA and Fisher’s PLSD (p < 0.05).

Results. Control values ranged from 45.1 MPa for Pertac II to 71.5 MPa for APX. Treatment 1 caused significant reduction in resin-resin bond strength for all materials tested but for two of the materials in treatment 2. Pertac II was the only material that did not show a statistical difference from control group for treatment 3. Treatment 4 re-established the control values for Z-250 and Renew and treatment 5 was the only one to show no statistical difference for all materials tested. SEM observation revealed a smooth surface in treatment 1, but treatment 3 showed a few craters. Treatment 4 and 5 showed a mixture of cohesive failure in the composite and adhesive.

Significance. The most reliable method for decontaminating saliva from resin surfaces involves the application of adhesives.

Introduction
The increasing popularity of dental composites has drawn attention to the importance of moisture and contamination control, because composites do not ‘forgive’ contamination or produce a better seal.
with time as amalgam restorations do. The difficulty of achieving moisture control is a common problem encountered in restorative dentistry, especially when rubber dam isolation is impossible. In addition, clinicians are encouraged to place resin composite restorations in increments to ensure complete polymerization of large restorations for optimal physical properties and bonding to the surrounding tooth structure. Gap formation between bonded restoration and tooth walls can be caused from the stresses of polymerization shrinkage.

Although the incremental filling technique is desirable, the longer time required for placement and polymerization of increments also can make contamination control more difficult. A few studies have been done on adhesives involving contamination of enamel and dentin surfaces; however, there is a lack of information on the effect of contamination between the surfaces of resin increments.

Many 'one-bottle' dental adhesives have become available in the past few years. They combine the primer and adhesive components of multi-step bonding systems in a single solution. Solvents such as acetone or ethanol in the one-bottle adhesives remove residual moisture and enhance resin wetting of the substrate. 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 'All-in-One' self-etching adhesives that combine etchant, primer, and adhesive in a single solution-have become available recently. There is a need to evaluate the role of these various new dentin bonding systems in decontaminating resin increments that were contaminated with saliva during placement of composite restorations.

The purpose of this study is to evaluate the effects of saliva contamination on microtensile bond strength (μTBS) between resin interfaces and to determine the best decontamination method to re-establish the original resin-resin bond strength. The hypothesis tested was that saliva 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 bonding agents were used. The bonding agents, compositions, batch numbers and manufacturers are listed in Table 1. An ethanol-based adhesive, Single Bond (SB), and an acetone-based adhesive, One-Step (OS) 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 used. Each bonding agent was used with its respective hybrid resin composite from the same manufacturer. SB was used with zirconia silica-filled Z-250, OS with barium glass-filled Renew, SE with barium glass-filled Clearfil APX and LP with quartz-filled Pertac II (Table 2). The bonding agents were applied and cured according to the manufacturers’ instructions (Table 1). A transparent vinyl cylinder 4 mm high x 10 mm wide was filled with resin composite. The excess was removed with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of the bonding agents.</th>
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<tbody>
<tr>
<td><strong>Adhesives</strong></td>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>Single Bond</td>
<td>Bis-GMA, HEMA, dimethacrylates, polyalkenoic copolymer, ethanol, water, photoinitiator</td>
</tr>
<tr>
<td>One-Step Clearfil SE Bond primer</td>
<td>Bis-GMA, BPDM, HEMA, acetone MDP, HEMA, hydrophilic dimetharylate, n,n Diethanol-p-Toluidine, camphoroquinone (CQ), water</td>
</tr>
<tr>
<td>Clearfil SE Bond adhesive</td>
<td>MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, n,n Diethanol-p-Toluidine, silanated colloidal silica CQ</td>
</tr>
<tr>
<td>Prompt L-Pop</td>
<td>Water, methacrylated phosphoric acid esters, fluoride complex w/ zinc, parabens</td>
</tr>
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Abbreviations: Bis-GMA: bisphenol-A-glycidylmethacrylate; HEMA: hydroxyethyl methacrylate; BPDM: bisphenyl dimethacrylate; MDP: 10-methacryloyloxy methacrylate.

Bonding procedures as executed in treatments 4 and 5: (a) apply primer, (b) mild air flow, (c) apply adhesive (d) light cured.
a spatula 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-curing unit (Optilux 501, Demetron/Kerr, Danbury, CT, USA). The intensity of the light was maintained at 690 mW/cm². The oxygen-inhibited layer was retained in order to replicate the clinical circumstances of incremental filling technique.

The composite cylinders were randomly divided into six groups according to the different surface contamination/treatments. Four cylinders were fabricated per treatment for each material, for a total of 96 composite cylinders. Saliva was collected from a single individual, at the site and at the same time that the specimens were made. Fresh saliva is considered an acceptable material to be used in saliva contamination testing.13,16 The specimens were treated as follows:

- **Control group.** The control group was not contaminated with saliva nor treated with bonding agent; rather, two layers of composite were added to the original cured composite.
- **Treatment 1.** Saliva was applied to the specimens with 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 saliva on top of the samples.

- **Treatment 2.** Saliva was applied with a microbrush and was immediately blown off with a strong stream of air from a distance of 1 cm for 20 s.

- **Treatment 3.** Saliva was applied and dried as previously described in Treatment 1. A copious amount of water was then used to rinse off the contamination for 20 s followed by air-drying for 20 s.

- **Treatment 4.** Saliva application and rinsing were done as in Treatment 3, except that a bonding agent was applied to the surface and was light-cured as recommended by its manufacturer (Table 1).

- **Treatment 5.** Saliva application was performed as in Treatment 1; however, instead of rinsing, a bonding agent was applied directly on the contaminated surface and was light-cured as recommended.

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.

The specimens were sectioned using a diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) into 0.7 mm 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 mm² ± 0.2 mm².17 The thickness and width of the bonded area of each specimen were measured using a digital micrometer. Each cylinder was cut into three specimens, for a total of 288 specimens (12 per material per treatment). The specimens were glued with cyanoacrylate (Zapit, DVA, Corona, CA, USA) to a Bencor Multi-T testing apparatus (Danville Engineering, Danville, CA) mounted in an Instron universal testing machine (model 4411, Instron Corporation, Canton, MA, USA). Specimens were loaded to failure at a crosshead speed of 1 mm/min. The microtensile bond strength was calculated by dividing the maximum load at fracture by the cross-sectional surface area of the bonded surface. If a 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 and Fisher’s PLSD (p < 0.05).

After debonding, the specimens were fixed in 10% neutral buffered formalin for at least 8 h for decontaminating the specimens. To evaluate the fracture pattern, three representative specimens from each treatment were chosen randomly and observed by two of the investigators. Both debonded sides of the fractured specimens were trimmed, placed on stubs, desiccated in ambient conditions, gold sputter-coated (5100 sputter-coater, Polaron...

### Table 2
Composition of the resin composites.

<table>
<thead>
<tr>
<th>Resin composites</th>
<th>Composition</th>
<th>Filler (%vol.)</th>
<th>Batch no.</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-250</td>
<td>Bis-GMA, UDMA, Bis-EMA</td>
<td>Zirconia-silica (60%)</td>
<td>0EH</td>
<td>3M ESPE, St Paul, MN</td>
</tr>
<tr>
<td>Renew</td>
<td>Bis-GMA, Triethylene glycol, BPDM</td>
<td>Barium-glass (59%)</td>
<td>0000007094</td>
<td>BISCO Inc., Schaumburg, IL</td>
</tr>
<tr>
<td>Clearfil APX</td>
<td>Bis-GMA, TEGDMA, CQ</td>
<td>Barium-glass (70%)</td>
<td>00551A</td>
<td>Kuraray Co., Ltd, Tokyo, Japan</td>
</tr>
<tr>
<td>Pertac II</td>
<td>No data</td>
<td>Quartz-filled</td>
<td>012, 014</td>
<td>3M ESPE, St Paul, MN</td>
</tr>
</tbody>
</table>

Abbreviations: BisGMA: bis-phenol-A-diglycidyl methacrylate; UDMA: urethane dimethacrylate; bis-EMA: bisphenol A polyethylene glycol diether dimethacrylate; BPDM: bipheno dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; MDP: 10-methacryloyloxy methacrylate.
Equipment Ltd, Watford, England), and examined with 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 treatments.

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). Fisher’s PLSD revealed no statistical difference between treatment 5 and the control group (p = 0.1355) but all other treatments had significantly lower microtensile bond strengths than the control (p < 0.05).

One-way ANOVA and Fisher’s PLSD test revealed that slowly drying the saliva on top of the specimens (treatment 1) resulted in significant reductions in resin–resin bond strengths for all materials (p < 0.0001), ranging from 16.4% (Z-250) to 43.4% (APX) of the control values. Drying saliva forcefully on the surfaces (treatment 2) resulted a significant decrease in bond strength for Z-250 (p < 0.0001) and APX (p = 0.0012); however, bond strengths were not significantly different from the control treatments for either Renew (p = 0.8408) or Pertac II (p = 0.7592). Rinsing saliva off with water (treatment 3) produced a significant difference from the control for Z-250 (p = 0.0058), Renew (p = 0.0146) and APX (p = 0.0122), but not for Pertac II (p = 0.2461).

For Z-250 and Renew, adding their respective bonding agents (treatment 4) re-established bond strengths to nearly the control level (p = 0.5997 and p = 0.5965, respectively). However, bond strengths remained significantly lower than the controls for both APX (p = 0.004) and Pertac II (p < 0.0001). The application of bonding agent on top of slowly dried saliva (treatment 5) re-established bond strengths to values similar to the control values for all materials tested (p < 0.05).

Scanning electron microscopic observation revealed a smooth surface on the specimens in treatment 1 when compared to the other treatments (Figs. 2 and 3). Treatment 3 specimens had differences in texture from control treatments.

Figure 1 Results of microtensile bond strength test for different restorative materials MPa (Bar = SD). Means with the same superscript are not statistically different within each composite/adhesive treatment (Fisher’s PLSD, p > 0.05).

Figure 2 (a) and (b) are representative SEM photographs of fractured specimen without contamination (Control group) × 2000. (a) Z-250. (b) APX.
a few craters or blisters indicating water, air, or saliva might still have been trapped in the composite buildup (Fig. 4). A mixture of cohesive failure in composite and adhesive in both base and first increment was observed under the SEM for treatments 4 and 5.

**Discussion**

Saliva is mostly water 99.4% with 0.6% solids. The solid is composed of macromolecules like proteins, glycoprotein sugars and amylase, inorganic particles like calcium, sodium and chloride and organic particles like urea, amino acids, fatty acids and free glucose. In this study, salivary contamination caused significant decreases in bond strength between resin increments, which is in agreement with results published by Hitmi et al. In their study, salivary contamination was achieved after polymerization of the bonding agent on dentin prior to application of composite.

Scanning electron microscopic observation of saliva-contaminated resins revealed a smooth surface on the specimens, (Figs. 2 and 3), which probably resulted in reduced bond strengths due to lack of interaction of the increment with the contaminated surface. Drying the saliva quickly resulted in significantly higher bond strengths than drying the saliva slowly, and this may be attributed to saliva film thickness. The water evaporates leaving a film of glycoprotein sugars on the surfaces. Although quick drying improved the bond strengths of two materials, this procedure may not be a completely reliable method for decontaminating the surface because Z-250 only showed re-establishment of 40% of control values, and APX showed 75%.

Rinsing saliva with water before adding new layers of composite raised the resin–resin bond strength to 77–80% of control values for Z250, Renew and APX and to the control values for Pertac II. SEM observation of treatment 3 showed a few craters or blisters indicating water, air or saliva might still have been trapped in the composite buildup (3). The bond strength data, along with the SEM photographs (Fig. 5), suggest that once saliva contacts the composite, salivary pellicle deposits on the composite and lowers the bond strength of the next layer, even if the saliva is there for only a short time (treatment 2) or is rinsed away with water (treatment 3), indicating that this approach...
is not a predictable method for decontaminating the surfaces.

The addition of a bonding agent in treatment 4 increased the bond strength of Z-250 and Renew to similar levels as their controls. Z-250, Renew and APX all had bond strengths approaching 60 MPa. A mixture of cohesive failure in composite and adhesive in both the base and the first applied increment was observed under the SEM.

In 1985, Lloyd and Dhuru\textsuperscript{19} made repairs to a day-old composite and found that inclusion of Scotch-bond (3M) bonding agent raised the fracture toughness of P10 (3M) specimens kept in saliva for 1 h back to values obtained for immediately repaired non-contaminated specimen. Shahdad and Kennedy\textsuperscript{20} reported that surface abrasion significantly improved the bond strength and that the use of bonding agents further enhanced bond strength but not significantly. They also reported that the use of the bonding agent alone improved the bond strength significantly ($p < 0.001$). Fritz et al.\textsuperscript{8} tested an experimental adhesive resin containing UDMA, HEMA and 4-META dissolved in acetone on saliva-contaminated enamel and dentin surfaces. They applied the saliva after curing the surfaces, rinsed it off and applied resin composite with or without primer. They found no difference between these treatments and a statistical significant difference from control treatment for dentin. This is in disagreement with our results for treatment 4 and shows that more research is necessary in this area to determine the effect on all the different surfaces of the cavity preparation.

Interestingly, application of a bonding agent on the dried saliva-contaminated surface gave the most reliable results in all treatments. This was especially noted with the self-etching primer/adhesive. The single-bottle adhesives contain solvents such as ethanol (SB) and acetone (OS) that seem able to denature the glycoprotein sugars and remove the saliva contamination from the surface to create bond strength similar to the rinsed surfaces in treatment 4. A similar phenomenon was observed with the self-etching primer (SE), which contains derivatives of ethanol. Surprisingly, Prompt L-Pop re-established only 53% of bond strength of the control values for Pertac II in treatment 4 but 100% in treatment 5 (Fig. 1). These results may have been caused by moisture trapped on the composite surface interfering with the bonding ability or by failure of the bonding agent to form a uniform surface coating. It has been reported that Prompt L-Pop might not be successfully polymerized in thin layers,\textsuperscript{21} 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.\textsuperscript{22,23} The solvent in Prompt L-Pop is water and the difference between treatments 4 and 5 may also be explained by the non-volatile nature of this solvent compared to the other dentin bonding agents. More careful drying is necessary to ensure complete removal of the water solvent. El-Kalla and Garcia-Godoy\textsuperscript{5} tested the effect of saliva on etched enamel and dentin on bond strengths using three adhesives containing acetone and one containing water as a solvent. The water-based bonding agent was the only one significantly different from its control treatment when tested on enamel but showed a non-significant difference when tested on dentin.

Webster et al.\textsuperscript{9} found that if the saliva contamination occurred after a primer had been placed and cured on enamel, reapplication of primer after drying the saliva provided a good bond to the underlying primer layer. Rosa et al.\textsuperscript{6} spread saliva on acid-etched enamel and dentin on bond strengths using three adhesives containing acetone and one containing water as a solvent. The water-based bonding agent was the only one significantly different from its control treatment when tested on enamel. Application of saliva lowered the bond strength statistically only

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure_5.png}
\caption{(a) and (b) are representative SEM photographs of fractured specimens that were contaminated with saliva, dried and rinsed with water, dried again and bonding agent applied showing mixture of cohesive failure in composite and adhesive in both the base and the first applied increment (Treatment 4) × 2000. (a) Z-250/S B. (b) APX/SE.}
\end{figure}
for Scotchbond Multi-Purpose, which was explained by a high percentage of organic solvents in SB and Prime&Bond NT, but no such solvents are in Scotchbond Multi-Purpose. The adsorbed moisture of saliva may be rapidly displaced if the solvent is sufficiently hydrophilic. Xie et al. also showed decreased bond strength when he applied the adhesive from Scotchbond Multi-Purpose after contaminating etched enamel with artificial saliva. Re-etching restored the bond strengths. In a similar study by Rosa et al. the same adhesives were tested on the effect of saliva contamination on dentin and found no statistically significant difference within each adhesive. In that case they also used the primer from Scotchbond Multi-Purpose, which contains HEMA, water, and polyalkenoic acid which facilitates bonding to wet dentin. Hitt and Feigal and Feigal et al. have reported that the bonding agents containing hydrophilic monomers can counteract the adverse effect of saliva on the bond strength of sealants to etched enamel. This result is consistent with our finding that priming the saliva-contaminated resin composite yielded the highest mean bond strengths and may be related to the fact that the resin was light-cured before the contamination occurred. Also, its hydrophobic nature might prevent the saliva from diluting or penetrating its surface. Peshke et al. tested the influence of incorrectly performed steps during the application of the water-based dentin adhesive system OptiBond FL on the marginal quality of Class V restorations in vitro and found that saliva contamination prior to application of the primer solution did not affect the margin quality significantly.

To simulate in vivo manipulation of composite surfaces prior to re-bonding, 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 complex stresses during testing, the technique adopted for this study was chosen because it more closely paralleled the clinical situation. The oxygen inhibited layer is viscous and contains unreacted acrylate groups which improve adhesion between the substrate and the second layer by the formation of covalent bonds. This unreacted layer may, however, induce brittleness because of inadequate links due to a local decrease in photo initiator concentration. Truffer-Boutry reports higher shear bond strength from the oxygen-inhibition on the surface layer of the substrate compared to surfaces without oxygen-inhibition. The methodology used in this study maintained the oxygen-inhibited layer in a way similar to incremental filling technique. Since 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.

Conclusions

Saliva contamination significantly reduced bond strengths between resin composite surfaces regardless of the materials evaluated. Blowing the saliva off quickly or rinsing with water did not restore bond strengths to normal levels for some of the materials tested. SEM examination suggested that water, air or saliva might remain trapped between resin layers. Within the limitations of this study, it can be concluded that the application of a dentin/enamel adhesive is necessary whenever saliva contamination exists on composite increments to ensure better interfacial bonding. Rinsing the saliva from the surfaces might not be necessary to promote good resin-resin adhesion.

Acknowledgements

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References