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## **Effect of Saliva Contamination on the Bond Strength of Dentin Adhesives to Central and Peripheral Primary Dentin *in vitro***

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### **ABSTRACT**

The bond strength of adhesives to dentin have been shown to be affected by a number of different factors, including intrinsic properties of the prepared dentin, various types of contamination and the chemical composition of the adhesive agent. The present study investigates the microtensile bond strength ( $\mu$ TBS) of two different bonding systems at different dentinal areas of primary dentin after saliva contamination. Caries-free primary molars were randomly divided into four groups (n = 10) for  $\mu$ TBS. Prime and Bond NT (etch-and-rinse) and Clearfil Protect Bond (two step self-etch) adhesives were tested under the following conditions: (a) control, (b) contamination with saliva prior to adhesive application. Following adhesive and composite superstructure application  $\mu$ TBS was measured. Maximum load at failure (N) was recorded and converted to MPa. Statistical analysis was carried out using one-way ANOVA with Tukey's test. No statistically significant difference was found between the  $\mu$ TBS of the contaminated and control groups in the central region of primary dentin for either adhesive system tested ( $p > 0.05$ ). However, saliva contamination resulted in significant reductions in bond strength in the peripheral region ( $p < 0.05$ ) for both adhesive systems tested. In the saliva contaminated groups,  $\mu$ TBS was higher in the central region than in the peripheral region. The etch and rinse adhesive performed better than the two-step self-etching adhesive under saliva contamination in both the peripheral and central regions of primary dentin. Results indicate that saliva contamination should be avoided when restoring primary teeth with proximal cavities using both Prime and Bond NT (etch and rinse) and Clearfil Protect Bond (two step self-etch) adhesives. However, confirmatory studies are needed before conclusive recommendations can be made for clinical practice.

**Key words:** Dentin adhesives, microtensile bond strength, primary tooth dentin, saliva contamination, adhesive systems, dentin area

### **INTRODUCTION**

A rise in aesthetic expectations and improvements in bonding systems have led to the widespread use of resin-based bonding systems for the restoration of primary teeth (Pashley *et al.*, 1982; Powers *et al.*, 2003). The bond strength of adhesives to dentin have been shown to be affected by a number of different factors, including intrinsic properties of the prepared dentin (e.g., depth, tubule diameter, morphology, calcium concentrations), various types of contamination (gingival fluid, blood, saliva, hand-piece oil) and the chemical composition of the adhesive agent (Fritz *et al.*, 1998; Prati and Pashley, 1992; Van Meerbeek *et al.*, 2003). The two main alternatives

currently used for dentin bonding are etch and rinse and self-etch adhesives (Hitmi *et al.*, 1999). The latter are particularly attractive in pediatric dentistry, since, they require fewer steps and less time, which helps to avoid contamination of the operative field (Sattabanasuk *et al.*, 2006). However, many carious lesions in primary teeth are located in areas that are difficult to isolate, especially near or at the gingival margin, where saliva contamination is more likely to occur (Tagami *et al.*, 1990). The effect of saliva contamination on the bond strength of adhesive systems to dentin is controversial. Several studies (Jacobsen and Soderholm, 1995; Van Meerbeek *et al.*, 2003, 2010) have shown saliva contamination to significantly reduce the bond strength of dentin adhesives, while others have reported no such reductions (Gwinnett, 1992; Humphrey and Williamson, 2001). Moreover, there is no consensus about the relationship between dentin region and bond strength (Fritz *et al.*, 1998). The clinical performance of adhesive restorations is affected by the strength of the bond between the adhesive agent and dentin, making it important to determine the effects of saliva contamination on bond strength, however, there is no study to date exploring the effects of saliva contamination on adhesive bond strength to different sites of primary dentin. Therefore, this study aimed to evaluate the  $\mu$ TBS of two different bonding agents at different dentinal areas after saliva contamination.

**MATERIALS AND METHODS**

**Tooth preparation:** Details of the materials and application procedures tested are given in Table 1. The study was conducted using 40 caries-free human primary second molars that were exfoliated or extracted for orthodontic reasons. Teeth were stored in distilled water at 4°C for a maximum period of three months before use (Kitasako *et al.*, 2000). Teeth were cleaned of debris and embedded in an acrylic mold to 2 mm below the cervical line for adaptation to a microcut device. The occlusal surfaces were sectioned perpendicular to the long axis of the tooth using a low-speed diamond saw under water cooling in order to expose superficial dentin within 1-2 mm of the Dentino-enamel Junction (DEJ). Exposed dentin surfaces were inspected with a light microscope (Olympus SZ61, Tokyo, Japan) to ensure that no enamel remained. A uniform, flat dentin surface and smear layer were created by abrading each specimen with 600-grit carbide paper under water (Hosoya, 1994).

Table 1: Composition and application mode of adhesive systems used In this study

Adhesive systems	Composition	Mode of application
<b>Prime and Bond NT</b>		
Batch# 070212 Dentsply De Trey Konstanz, Germany	PENTA, UDMA, acetone, nano-filler, cetyaminehydrofluoride, initiators, stabilizer	Apply etchant (37% phosphoric acid) to dentin 15 sec, gently air dry, apply adhesive to the prepared surfaces with a brush for 20 sec, air dry for 5 sec, light cure 20 sec
<b>Clearfil Protect Bond</b>		
Batch# 41124 Kuraray Medical Inc., Japan	Primer: MDP, MDPB, HEMA, water Bond: MDP/Bis-GMA/HEMA/ camphorquinone/colloidal silica/NaF	Apply primer to the prepared surfaces for 20 sec, gently dried for 5 sec, apply one coat of adhesive and gently air dry for 5 sec and light cure 10 sec

PENTA: Dipentaerythritol penta acrylate monophosphate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4META: 4-methacryl oyl oxethyl trimellitate anhydride, MDPB: 12-methacryloyloxy-dodecylpyridinium bromide, UDMA: Urethane dimethacrylate, Bi-GMA: Bisphenol glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate

**Experimental design:** Teeth were then randomly distributed into four groups of 10 teeth each and prepared as follows:

- **Group 1:** Prime and Bond NT (Dentsply/DeTrey Konstanz, Almany) (saliva contaminated group). Dentin surfaces were acid-etched with 36% phosphoric acid gel (Scotchbond Etching Gel, 3M ESPE, St. Paul, MN, USA) for 15 sec, thoroughly washed and gently air dried for 2 sec and contaminated with 0.01 mL of fresh human saliva 30 collected from a single donor and applied with a micropipette. Saliva was left undisturbed for 10 sec (Sattabanasuk *et al.*, 2006) and the contaminated dentin was then gently dried for 10 sec from a distance of 1 cm. (Jacobsen and Soderholm, 1995) Prime and Bond NT dentin adhesive was applied according to the manufacturer's instructions (Table 1). A hybrid resin composite material (TPH, De Trey/Dentsply, Konstanz, Germany) was used to prepare a superstructure of approximately 5 mm in height to provide sufficient bulk for microtensile bond-strength testing. Resin composite was applied in 2-3 layers and each layer was cured for 40 sec
- **Group 2:** Prime and Bond NT (control group). Dentin surfaces and superstructures were prepared as in group 1, but without saliva contamination
- **Group 3:** Clearfil Protect Bond (Kuraray America, New York, USA) (saliva contaminated group). The primer of this two-step dentin adhesive system was applied to the dentin surfaces according to the manufacturer's instructions. Dentin surfaces were contaminated as in group 1. Following contamination, the dentin bonding agent was applied according to the manufacturer's instructions (Table 1) and superstructures were created using a resin composite (Clearfil AP-X, Kuraray Medical Inc., Japan), as in group 1
- **Group 4:** Clearfil Protect Bond (control group). Dentin surfaces and superstructures were prepared as in group 3, but without saliva contamination

**Measurement of microtensile bond strength:** Teeth were visually assessed and marked with indelible ink to identify the central region (between the pulp horns) and the peripheral region (between the pulp horns and the DEJ) and were then stored in distilled water at 37°C for 24 h.

Teeth were then sectioned using a water-cooled low-speed Isomet 1,000 diamond micro-slicing saw (Buehler, Lake Bluff, IL, USA) across the adhesive interface along the x and y axis to obtain stick-shaped specimens with a bonding area of  $1\pm 0.2$  mm<sup>2</sup>. No failures occurred during specimen production.

The beam-shaped specimens were fixed by their ends to a microtensile bond-strength testing device (Force Gauge 200 X 0.2 N, Scales Galore, A Division of Itin Scale Co., Inc. 431 Avenue Brooklyn, USA) using a cyanoacrylate adhesive (502, Eva Bond group, Japan) and tested in tension at a crosshead speed of 1.0 mm min<sup>-1</sup>. Bond strength (MPa) for each specimen was calculated as the failure load (N) divided by the cross-sectional area of the bonded interface.

**Statistical analysis:** Shapiro-Wilk test was applied and showed a normal distribution of data. Mean  $\mu$ TBS of the groups were calculated using one way analysis of variance (ANOVA) and Tukey's test was used to identify significant differences between group, with the level of significance set at  $p < 0.05$  (Table 2). Statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

## RESULTS

Means and standard deviations of microtensile bond strength values (MPa) for superficial central dentin and superficial peripheral dentin are given in Table 2. In the central region, no

Table 2: Mean micro tensile bond strengths (MTBS) and standard deviation for a two-step etch and rinse adhesive (Prime and Bond NT), a two-step self etch adhesive (Clearfil Protect Bond) and all in one dentin adhesive (I bond) to superficial peripheral and superficial central dentin

Groups	MTBS (SD), MPa	
	Superficial peripheral dentin	Superficial central dentin
1: Prime and Bond NT Saliva contaminated	24.2 (11.1) <sup>a1</sup>	36.9 (17.4) <sup>b1</sup>
2: Prime and Bond NT control	38.4 (17.4) <sup>a2</sup>	38.1 (29.9) <sup>a1</sup>
3: Clearfil Protect Bond Saliva contaminated	15.9 (10.9) <sup>a1</sup>	29.1 (16.9) <sup>b1</sup>
4: Clearfil Protect Bond control	26.2 (12.4) <sup>a3</sup>	42.3 (25.7) <sup>b1</sup>

Differences in superscript letters indicate statistically significant differences within columns and differences in superscript numbers indicate significant differences within rows at  $p < 0.05$

statistically significant differences were found between the  $\mu$ TBS to superficial primary dentin of the saliva-contaminated and control groups for either adhesive system tested ( $p > 0.05$ ). However, in the peripheral regions, saliva contamination significantly reduced the  $\mu$ TBS of both tested adhesives to peripheral superficial primary dentin ( $p < 0.05$ ). Whereas the non-contaminated Prime and Bond NT etch and rinse adhesive specimens (group 2) had a mean  $\mu$ TBS of  $38.4 \pm 17.4$  MPa to peripheral dentine, the contaminated Prime and Bond NT specimens (group 1) had a mean  $\mu$ TBS of only  $24.2 \pm 11.1$  MPa. Similarly, the non-contaminated Clearfil Protect Bond two-step adhesive specimens (group 4) had a mean  $\mu$ TBS of  $26.2 \pm 12.4$  MPa, compared to only  $15.9 \pm 10.9$  MPa for the contaminated specimens (group 3).

Prime and Bond NT had significantly higher mean  $\mu$ TBS than Clearfil Protect Bond in saliva contamination groups of both region.

Stereomicroscope evaluation of samples showed mainly adhesive fractures in all groups.

## DISCUSSION

The present study examined the effect of saliva contamination on the  $\mu$ TBS of two adhesive systems to peripheral and central superficial primary dentin. Saliva contamination was found to decrease the  $\mu$ TBS of both adhesive systems to peripheral dentin, but did not affect the  $\mu$ TBS of either system to central dentin.

The search for restorative materials with improved adhesive capacity has been the object of considerable research in recent years. Evaluating bond strength to dental hard tissue is an important element in developing a better understanding of the clinical performance of bonding systems (Phrukkanon *et al.*, 2003). However, there is insufficient information currently available on the effects of saliva contamination on adhesive bond strength to different regions of primary tooth dentin. Contamination by saliva, blood and gingival crevicular fluid is a major clinical problem encountered during restorative dental treatment, especially when the cavity margins are near or at the gingival margins. While the problem of contamination can be exacerbated by a lack of cooperation in small children, which precludes the use of a rubber dam for isolation, resin-based materials are considered innately susceptible to dentinal moisture contamination, which has been shown to adversely affect bonding properties (Taskonak and Sertgoz, 2002; Van Meerbeek *et al.*, 2003).

The complex biological nature of dentin may affect the  $\mu$ TBS of adhesives in different ways (Marshall *et al.*, 1997). For example, the number of dentin tubules and location of the bonding area may alter the  $\mu$ TBS of adhesives (Finer and Santerre, 2004). Pashley (1989) reported that while tubule density and peritubular dentin area decreases with distance from the pulp, intertubular

dentin area increases with distance from the pulp. Previous studies (Hansen and Munksgaard, 1989; Hosoya, 1994; Staehle, 1999) have shown that dentin bond strength gradually decreases from the superficial to the deeper layers of dentin due to the decrease in intertubular dentin ratio. Standardization of dentin is difficult in primary molars due to the wide coronal pulpal chamber relative to the outer diameter of the tooth. In this study, standardization was achieved by removing occlusal enamel to a depth of 2 mm apical to the occlusal pit and using the superficial dentin only (Nikaido *et al.*, 1998).

Variations in dentin structure and composition occur not only with differences in depth, but from region to region as well. When compared to central dentin, peripheral dentin has fewer dentin tubules and they are oriented oblique or perpendicular to the long axis of the tooth. In theory, resin tags are unable to form in areas where the dentin tubules are located perpendicular to the long axis of the tooth (Pashley *et al.*, 1998). Gwinnett (1992) has shown that  $\mu$ TBS is negatively affected by resin infiltration into both the dentin tubules and the intertubular matrix in areas where the dentin tubules are exposed perpendicular to their long axis. A study by Cabrera and Macora (2007) that looked at the  $\mu$ TBS of resin-based composites to gingival, central and incisal enamel showed that  $\mu$ TBS decreased significantly with increases in the distance from the centre of the curing mass. In the present study,  $\mu$ TBS in the central region was higher than in the peripheral region for both adhesive systems in the saliva contaminated groups. In theory, proper bonding requires the shrinkage vectors of resin-based restorations to be oriented towards the bonded interfaces (Pashley, 1989) so that the resin composite is pulled away from the peripheral zones during polymerization (Van Meerbeek *et al.*, 2000). In the present study, reductions in bond strength in the peripheral region can be attributed to reduced dentinal tubule density as well as the orientation of shrinkage vectors from the peripheral to the central zones. Saliva is a very dilute solution composed of more than 99% water as well as immunoglobulin, glycoprotein, enzymes, mucins, nitrogenous products and a variety of electrolytes. Excess water from saliva had been reported to cause over wetting of dentin surfaces and reduce the bond strength of dentin adhesives. Salivary glycoprotein may also be absorbed and accumulate on the bonded surface, thus, interfering with proper adhesion and high-molecular-weight macromolecules in saliva may diffuse into the dentin tubules (Cabrera and Macora, 2007; El-Kalla and Garcia-Godoy, 1997; Hashimoto *et al.*, 2006) and complete with hydrophilic monomers during the hybridization process, causing a reduction in bond strength. Finally, enzymes in human saliva have been shown to degrade the Bis-GMA in composite and this hydrolytic activity may also contribute to the breakdown of the bonded interface (Hiraishi *et al.*, 2003; Park and Lee, 2004).

Studies examining the effects of saliva contamination on bond strength have had conflicting results. Fritz *et al.* (1998) claimed that saliva contamination decreased the  $\mu$ TBS of one-bottle, self-etch dentin adhesives to dentin by an average of 50%, whereas, Hansen and Munksgaard (1989) found that saliva contamination did not effect the shear bond strength of one-bottle, self-etch adhesives. Similarly, a study by El-Kalla and Garcia-Godoy (1997) reported no differences in the bond strength of one-bottle adhesives to either contaminated or non-contaminated dentin surfaces. Park and Lee found that saliva contamination reduced the bond strength of both two-steps, self-etch and etch and rinse adhesive systems to dentin. In the present study, saliva contamination was also found to significantly reduce the  $\mu$ TBS of both a two-step, self-etch adhesive and an etch and rinse adhesive to superficial primary dentin in the peripheral region; however, saliva contamination resulted in only a slight, insignificant reduction in  $\mu$ TBS to superficial primary dentin in the central region. Prior to this study, no clear differences

have been demonstrated in regional bond strength values under saliva contamination, especially for primary tooth dentin. In the present study, saliva contamination was blot-dried after conditioning in group 1 and after primer application in group 3. This means that the water filled collagen layer will collapse and that a dried protein film will be adsorbed to the dentin surface. The protein adsorbing properties of dentin have been reported previously (Cabrera and Macora, 2007). When blot-dried, the protein components of saliva will form a film on the dentin surface and these proteins will be adsorbed by the collapsed collagen (Cabrera and Macora, 2007). Both the collapse of the collagen layer and the protein film will prevent adhesive from penetrating the exposed collagen network and forming a sound hybrid layer. The findings of the present study showing lower adhesive bond strengths in the peripheral region when compared to the central region of the saliva-contaminated groups indicate that this mechanism would have a greater effect in the peripheral region of primary tooth dentin than in the central region. This result may be due to the regional differences of bonding strength would change with the property of adhesive materials.

The present study found that the etch and rinse adhesive system tested (Prime and Bond NT) performed better than the two-step, self-etching adhesive system tested (Clearfil Protect Bond) in both the central and peripheral regions when saliva contamination was present. It is possible that an increase in dentin wetness due to the presence of saliva inhibits the ability of water-based adhesives (such as Clearfil Protect Bond) to evaporate as easily and completely as ethanol and acetone-based adhesives (such as Prime and Bond NT) and poor evaporation and thus retention of water may result in a mechanical weakening of the adhesive layer and hence lower bond strengths. Previous studies have also found etch and rinse adhesive systems to exhibit higher bond strengths than self-etch adhesive systems (Can Say *et al.*, 2006; Senawongse *et al.*, 2004).

In conclusion, this study found saliva contamination to have a significant negative affect on the bond strength of adhesives to peripheral primary dentin, but not to central primary dentin. When saliva contamination was present, the etch and rinse adhesive system tested performed better than the two-step self-etching adhesive system tested in both regions. Results indicate that saliva contamination should be avoided when restoring primary teeth with proximal cavities using both Prime and Bond NT (etch and rinse) and Clearfil Protect Bond (two step self-etch) adhesives. However, confirmatory studies are needed before conclusive recommendations can be made for clinical practice.

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