

SELF-ETCHING PRIMER: THE EFFECT OF CONTAMINATION WITH A MIXTURE OF SALIVA AND BLOOD ON BONDING METALLIC BRACKETS

ABSTRACT

PURPOSE: To evaluate the shear bond strength (SBS) of metallic brackets bonded with Transbond Plus Self-Etching Primer (TPSEP) and Transbond Plus Color Change (TPCC) under contamination with a mixture of saliva and blood. **MATERIALS AND METHODS:** 42 human premolars were randomly divided into 2 groups (n=21). Group 1 (G1) were bonded under no contamination, and Group 2 (G2) was contaminated with saliva/blood before bonding. Both groups were bonded according to the manufacturer's instructions and were cleaned with pumice before bonding. The shear bond strength (SBS) tests were performed after 24 hours in distilled water at 37°C and after thermocycling. **RESULTS:** Both groups showed a homogeneous distribution in the Levene's test ($p>0.05$). The main shear bond strength value of G1 was 8.89 MPa with a standard deviation of 2.27; the value for G2 was 6.00 MPa with a standard deviation of 2.62 MPa. There was a significant difference between G1 and G2 (t-student test $p<0.05$). IRA scores indicated that the main value was IRA 0, and no significant difference ($\alpha=0.05$) was found between the groups. **CONCLUSION:** Contamination of blood mixed with saliva significantly decreases the shear bond strength, but even so, the performance is clinically acceptable.

PERES, Bernardo Urbanetto*
DEGRAZZIA, Felipe Weidenbach**
GHERS, Renésio Armindo***
ARRAIS, Fabíola Rossato****
FERRAZZO, Vilmar Antonio***

KEYWORDS

Adhesives. Shear strength. Orthodontic brackets.

DDS, Private Clinic, Charqueadas, Rio Grande do Sul*

MSc, DDS, Private Clinic, Santa Maria, Rio Grande do Sul**

PhD, MSc, DDS, Orthodontics, School of Dentistry, University of Santa Maria, Santa Maria, Rio Grande do Sul***

Pontifical Catholic of University of Rio Grande do Sul****

Correspondence: buperes@gmail.com (PERES BU) | Received 21 Jun 2013 Received in revised form 27 Jun 2013 Accepted 30 Jun 2013

INTRODUCTION

In the past, the concept of placing intra-oral appliances in orthodontics involved banding all teeth, resulting in poor hygiene, poor aesthetics, and discomfort to the patients. Usually, the treatment was long and expensive¹. Direct bracket bonding with composites through hybridization of dental tissues was first implemented in 1965. Nowadays, this technique is widely used and accepted among orthodontists; however, the bonding procedure requires a great deal of attention at each step and a contaminant-free surface.^{2,3,4,5,6}

Several factors can have a direct effect on the shear bond strength of brackets,⁷ such as saliva,⁸ blood,⁹ and water.¹⁰ Some dental surfaces have an inherent characteristic of not being able to be completely dry for bonding procedures, such as second molars that are not completely erupted and ectopic teeth during surgery traction procedures.

When an orthodontist faces such a scenario, the microporosities made by the acid etch are filled with fluids, decreasing the mechanical microretention and consequently, the surface energy.^{11,12,13} To overcome this issue, various hydrophilic adhesives exist, such as Transbond Plus Self-Etching Primer (TPSEP 3M, Unitek, Monrovia, EUA) and Transbond Plus Color Change (TPCC 3M, Unitek, Monrovia, EUA), which, when combined, tolerate moisture conditions in a more efficient

way.¹³ Both materials have been used successfully on bonding metallic brackets compared to the acid-etch technique and have demonstrated an acceptable shear bond strength, even on dry or moist surfaces.¹²

In clinical situations, both saliva and blood are present, and it is difficult to separate their effects on bonding procedures that involve inherent contamination. Moreover, there are few studies that examine the use of TPSEP and TPCC with an enamel surface that is contaminated by saliva and blood. Thus, the main objective of this study is to evaluate the shear bond strength of metallic brackets bonded with TPSEP/TPCC under contamination of a mixture of blood and saliva.

MATERIAL AND METHODS

Forty-two humans premolars were extracted for orthodontic reasons, twenty-one of which were upper teeth and twenty-one of which were lower teeth. The teeth were stored in distilled water that was frequently replaced. Teeth were not submitted to chemical treatment and were free from caries, cracks, or fractures.

The crowns were cut using a bur and then embedded in polyvinyl chloride tubes (PVC, Krona, NBR 5648, 25x20) with self-curing acrylic resin (Clássico, São Paulo, Brasil), leaving the labial surface exposed. The specimens were listed and then randomly divided (Random Allocation Software)

between groups 1 (control) and 2 (contaminated). The same procedure was performed on the upper and the lower premolars. Two groups with 21 random samples (n=21) were thus defined.

Thereafter, the teeth were subjected to a prophylactic treatment with pumice-powder paste-water containing no fluoride, then rinsed with an air-water syringe for 15 seconds, and then dried with an air-water syringe (Kavo Dental Excellence). Group 1 (G1—control) moved directly on to the bonding procedure.

Group 2 (G2—contamination) was subjected to a contamination protocol, in which saliva was taken from one of the authors, who was directed to brush his teeth after a one-hour period of not eating anything.

The blood was also taken from one of the authors and was collected by means of a hypodermic needle. Next, the blood was stored in tubes of EDTA (Ethylenediaminetetraacetic acid) trisodium 5% (Vacuplast). Blood and saliva were mixed in equal amounts (ratio 1:1) in a dappen glass with a regular size microbrush (KG Sorensen). Once a homogeneous mixture of the two fluids was attained, it was applied on the bucal surfaces of the samples for 15 seconds.

The bonding procedure was performed by a trained operator adhering to the following sequence: 1) application of Transbond Plus Self-Etching Primer (TPSEP, 3M, Unitek, Monrovia, EUA) and 2) bracket bonding (MBT,

Gemini Metal Brackets, REF 119-150, 3M, Unitek) with Transbond Plus Color Change (TPCC, 3M, Unitek, Monrovia, EUA). The TPSEP was applied to the enamel in accordance with the manufacturer's instructions (5 seconds), and the samples were then light-cured for 10 seconds. The TPCC was applied to the bracket base and then placed in the center region of the bucal surface of the samples. A Gillmore needle was then placed in the central area of the bracket and exerted a force of 456,3 cN for 10 seconds. The excess of resin was removed with a probe n°5 (Millenium) and then light-cured with a Light Emitting Diode (LED, Radii, SDI, 1200 mw/cm²) for 10 seconds on each surface of the bracket.

Once all samples had been subjected to this treatment, they were stored in distilled water at 37°C. The specimens were then submitted to thermocycling at 5°C/55°C (500 cycles, 60 seconds each).

Once the thermocycling was completed, all of the samples were placed in the testing device (Odeme), and the shear bond strength test was performed by means of an international test machine (EMIC DL 2000). The speed was 1 min/mm in line with the instructions for ISO 2003. The operator running the test did not know which group was being tested.

The force producing failure was recorded in newtons and converted into megapascals by dividing the measured force

values by the mean surface area of the brackets (14.1 mm²).

The adhesive remnant index¹⁴ (ARI) was used to evaluate the amount of adhesive left on the enamel surface and to establish the sites of fracture. Subsequently, the bracket base was examined with a stereomicroscope at 10x magnification, and the remaining adhesive was scored as follows: an ARI of 0 means there was no adhesive on the enamel surface; an ARI of 1 indicates that there was less than 50% adhesive on the enamel surface; an ARI of 2 indicates more than 50% adhesive on the enamel surface; and an ARI of 3 means that 100% of the adhesive remained on the enamel.

The shear bond strength (SBS) values obtained were tabulated in spreadsheets and then analyzed by the software SPSS (Statistical Package for Social Sciences, versão 18.0). The normality distribution was verified using the Shapiro-Wilk test, and the equality of variances was assessed with the Levene's test. Both groups were compared by means of the T-Student test. The ARI were also listed in a spreadsheet and compared with the chi-square test ($\alpha=0.05$).

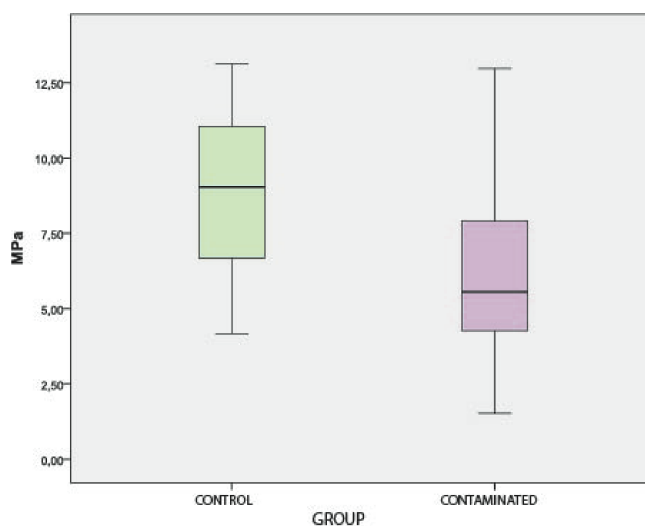
RESULTS

Descriptive statistics related to the SBS of both groups is represented in Figure 1 and Table 1. The mean SBS value for Group 1 (G1—Control) was 8.89 MPa with a standard deviation (SD) of 2.27 MPa. The mean for

Group 2 (G2—Contamination) was 6.00 MPa with a SD of 2.62 MPa. The data indicated a homogeneous distribution (Levene's Test $p>0.05$), and the T-Student test indicated a statistical difference between the groups ($p<0.05$).

The ARI scores (Table 2) for G1 were 47.6% (ARI 0), 19% (ARI 1), 19% (ARI 2), and 14.% (ARI 3). In the contamination group (G2), similar findings were observed, with 76% (ARI 0), 9.5% (ARI 1), 4.8% (ARI 2), and 9.5% (ARI 3). There was no statistical difference between the two groups (Chi-Square test, $\alpha=0.05$).

Figure 1 - Distribution of the shear bond strength between the two groups (G1—Control and G2— Contaminated).



DISCUSSION

Due to the large variability of methods used in in vitro studies to test shear bond strength of brackets, it can be challenging to discuss results.⁶⁻¹⁹

The literature describes several factors that can have a negative influence on bonding brackets. The presence of saliva, water, and blood is known as the main factor responsible for decreasing bond strength when using composite resins systems. To increase the efficacy of bonding on inherently contaminated surfaces, new materials have been developed, such as Transbond Plus Self-Etching Primer and Transbond Plus Color Change (3M, Unitek, Monrovia, EUA). The materials, when combined, create a moisture-tolerant bonding system. The first time that these materials were tested with water and saliva¹⁵ similar results were found in this study (dry: 6.93 ± 3.34 MPa and under contamination: 7.78 ± 4.45 MPa). However, the present study resulted in higher values for dry conditions (8.89 ± 2.27 MPa) and lower values when the

enamel was contaminated (6.00 ± 2.62 MPa). It should be emphasized that this experiment used saliva mixed with blood in a one-to-one proportion.

In clinical conditions, it is impossible to separate the contaminant fluids, which is why blood and saliva were used together in this study. In comparing blood and saliva to dry conditions, the blood seems to be a worse contaminant fluid, as it decreases the shear bond strength even more strongly. The advantage of using the two combined is that it mimics clinical conditions more closely; however, it is impossible to differentiate the different roles of the two contaminants, although blood has been characterized as a physical barrier that blocks micromechanical imbrication.^{8,12,20}

Table 1. Shear Bond Strength Values (MPa).

Groups	Main	Standard Deviation	Minimum	Maximum	Significant*
Control (G1)	8.89	2.27	4.16	13.12	A
Contaminated (G2)	6.00	2.62	2.65	12.97	B

*Equal letters indicate no statistical difference.

Table 2. Adhesive Remnant Index (ARI)*.

Group	ARI = 0	ARI = 1	ARI = 2	ARI = 3	Total
Control (G1)	10 (47.6%)	4 (19%)	4 (19%)	3 (14.3%)	21 (100%)
Contaminated (G2)	16 (76.2%)	2 (9.5%)	1 (4.8%)	2 (9.5%)	21 (100%)
Total	26 (61.9%)	6 (14.3%)	5 (11.9%)	5 (11.9%)	42 (100%)

*An ARI of 0 means that no adhesive was left on the tooth surface, 1 indicates that less than half of the surface of the bracket had adhesive, 2 means more than half of the surface of the bracket had adhesive, and 3 means that 100% of the adhesive was left on the enamel surface.

Recent studies^{9,21,22} demonstrate that once the enamel surface is contaminated with blood, a lower bond strength is observed, but the best results were those using TPSEP combined to TPCC. Under dry conditions, the results (8.89 ± 2.27 MPa) were similar to studies that found 9.91 ± 2.23 MPa.²² A situation in which blood was applied to the enamel resulted in 5.24 ± 2.45 MPa versus 6.00 ± 2.62 MPa found in the present paper. Although both studies used blood mixed with saliva, they differed in terms of the thermocycling process.

The results from Vicente et al.,¹⁵ which used TPSEP+TPCC under no contamination (6.93 ± 3.34 MPa) and then compared these results to using TPSEC+TPCC with saliva contamination (7.78 ± 4.45 Mpa), diverge from those of the present study. Cacciafesta et al.,²³ however, showed that there is no statistical difference between conditions in which TPSEP+TPCC is used in the presence of saliva contamination (7.25 ± 1.88 MPa) and with dry surfaces (10.31 ± 2.53 MPa). These differences lead us to conclude that when the surface is

contaminated with blood and saliva, even in equal proportions, the material behaves similar to circumstances of contamination by blood alone, once there is a more severe decrease in shear bond strength¹².

Pithon et al.¹⁶ found higher results with the use of Transbond Plus Self-Etching Primer,

combined with different resins, than the present study, although this fact can be explained by the use of different brackets and teeth (premolars versus incisors). Endo et al.¹⁰ and the present paper observed similar results; however, permanent teeth are compared with primary teeth. Primary teeth usually have a smaller prismatic layer and less minerals, which might be associated with the lower shear bond strength.

Oonsombat et al.²¹ showed that the moment of the contamination, before or after the application of the self-etching primer, had no influence on the shear bond strength. Since the main objective was to evaluate the material and how it relates to enamel, the present study introduced contamination before the TPSEP. Santos⁹ used TPSEP after contamination and also found a lower shear bond strength. A study²³ comparing different moments of contamination using saliva and water also revealed no difference related to when the samples were contaminated.

The use of blood as a contamination fluid in in vitro studies is also worthy of discussion since it is necessary to add anticoagulants to maintain the consistency of the fluid during the experiment.²¹ The majority of the studies that used blood do not clearly describe how the blood was taken, how it was stored, or the use of anticoagulant substances.^{12,20} The present paper used blood taken from

one of the authors, which was stored in Trissodium EDTA 5%.

Another methodological issue to be highlighted is the prophylaxis with pumice powder before the use of TPSEP. Although studies usually describe this procedure,^{16,21,20,23} they do not emphasize its importance for self-etching primers. The prophylaxis was recently compared in a random clinical trial,²⁴ and it was corroborated that this step was indispensable since there is no acid etching, rinsing, or drying to guarantee a clean enamel surface.²⁷

The specimens were thermocycled (500 cycles of 5°C and 55°C) 24 hours after storage in distilled water.²⁶ It is important to highlight the attempt to simulate clinical conditions; papers^{25,28} reporting a thermocycling effect demonstrated a significant decrease in shear bond strength when metallic brackets were used. Faltermeier et al.¹² submitted the samples to this process, which was not observed in other studies.^{8,10,16} Oztoprak²⁰ used bovine incisors and the same adhesive, but did not use thermocycling, which could explain the higher shear bond values in dry conditions (13.76 ± 2.76 MPa).

The present study, in contrast with the results presented by Daub,²⁸ resulted in similar values to those reported by Cunha²² (9.91 ± 2.23 MPa) and higher than those presented by Vicente¹⁵ (6.93 ± 3.34 MPa). It is important to note that neither^{22,15} used thermocycling.

The adhesive remnant index (ARI) revealed that the failure site was usually in the tooth, which could be explained by the contamination process or the superficial etching of the enamel by the self-etching primer^{15,16,18,29} (G1— 47.6% ARI=0 and G2— 76.2% ARI=0). However, this finding may indicate an advantage with regard to removing the remaining adhesive more easily, consequently shortening the debonding and rebonding procedure. In contrast with the results presented in this paper, Pithon et al.¹⁶ reported that under dry conditions, the majority of the samples had an ARI=2 (46.7%) and an ARI=3 (33.3%). In a random clinical trial²⁹ using TPSEP, the ARI results were similar to those of this study. Half of the contaminated teeth had an ARI=0. The second group (which was contaminated with blood mixed with saliva) had results similar to those of other studies that used similar methods.²²

Through the results of this study, the clinical use of this material (TPSEP+TPCC) on contaminated surfaces (fabricant purpose) must be carefully indicated. The difference in the mean shear bond strength between the two groups was statistically significant; however, both values (G1—8.89 MPa and G2—6.00 MPa) are capable of supporting orthodontic forces,²⁵ Nevertheless, it is not possible apply these results directly to the clinic, as the oral cavity is a very complex environment that

cannot be accurately reproduced in the laboratory.

CONCLUSION

- The presence of blood mixed with saliva significantly decreased the shear bond strength; however, the values are clinically acceptable;
- Contamination with blood and saliva had no significant effect on the adhesive remnant index.

REFERENCES

1. Bishara SE, Khowassah MA, Oesterle LJ. Effect of humidity and temperature changes on orthodontic direct-bonding adhesive systems. *J Dent Res.* 1975; 54: 751-758.
2. Newman GV. Epoxy adhesives for orthodontic attachments: Progress report. *Am J Orthod.* 1965; 51: 901-912.
3. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J. Dent. Res.* 1955; 34: 849-853.
4. Chuanga S, Changa C, Yamanc P, Changd L. Influence of enamel wetness on resin composite restorations using various dentine bonding agents. Part I—Effects on marginal quality and enamel microcrack formation. *J Dent.* 2006; 34:343-351.
5. Lina BA, Jaffer F, Duff MD, Tanga YW. Identifying enzyme activities within human saliva which are relevant to dental resin composite biodegradation. *Biomaterials,* 2005; 26:4259-4264.
6. Chung CWM, Yiu CKY, King NM, Hirashi N, Tay FR. Effect of saliva contamination on bond strength of resin luting cements to dentin. *J Dent.* 2009; 37:923-931.
7. Wickwire NA, Rentz D. Enamel pretreatment: A critical variable in direct bonding systems. *Am J Orthod Dentofacial Orthop,* 1973; 64:499-512.
8. Cacciafesta V, Sfrondini MF, Soribante A, Andellis MD, Kiersy C. Effects of blood contamination on the shear bond strengths of conventional and hydrophilic primers. *Am J Orthod Dentofacial Orthop.* 2004; 126: 207-212.
9. Santos BM, Pithon MM, Ruelas AC de O, Sant`Anna EF. Shear bond strength of brackets bonded with hydrophilic and hydrophobic bond systems under contamination. *Angle Orthod.* 2010; 80: 963-967.
10. Endo T, Ozoe R, Shinkai K, Shimomura J, Katoh Y. Comparison of shear bond strengths of orthodontic brackets bonded to deciduous and permanent teeth. *Am J Orthod Dentofacial Orthop.* 2008; 134: 198-202.
11. Rajagopal R, Padmanabhan S, Gnanamani J. A comparison of shear bond strength and debonding characteristics of conventional, moisture-insensitive, and self-etching primers in vitro. *Angle Orthod.* 2004; 74: 264-268.
12. Faltermeier A, Behr M, Rosentritt M, Reicheneder C, Mübig D. An in vitro comparative assessment of different enamel contaminants during bracket bonding. *Eur J Orthodont.* 2007; 29: 559-563.
13. Brauchli L, Eichenberger M, Steineck M, Wichelhaus A. Influence of decontamination procedures on shear forces after contamination with blood or saliva. *Am J Orthod Dentofacial Orthop.* 2010; 138: 435-441.
14. Artun JBS. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. *Am J Orthod Dentofacial Orthop.* 1984; 85: 333-340.
15. Vicente A, Mena A, Ortiz JA, Bravo LA. Water and saliva contamination effect on shear bond strength of brackets bonded with moisture-tolerant light cure system. *Angle Ortho.* 2009; 79: 127-132.
16. Pithon MM, Ruellas AC de O, Sant`Anna EF, Oliveira MV de, Bernardes LAV. Shear bond strength of brackets

- bonded to enamel with a self-etching primer. *Angle Orthod.* 2009; 79: 133–137.
17. Reicheneder CA, Gedrange T, Lange A, Baumert U, Proff P. Shear and tensile bond strength comparison of various contemporary orthodontic adhesive systems: An in-vitro study. *Am J Orthod Dentofacial Orthop.* 2009; 135: 422e1–422e6.
18. Scougall-Vilchis RJ, Ohashi S, Yamamoto K. Effects of 6 self-etching primers on shear bond strength of orthodontic brackets. *Am J Orthod Dentofacial Orthop.* 2009; 135: 424e1–424e7.
19. Zeppieri IL, Chung CH, Mante FK. Effect of saliva on shear bond strength of an orthodontic adhesive used with moisture-insensitive and self-etching primers. *Am J Orthod Dentofacial Orthop.* 2003; 124: 414–419.
20. Oztoprak MO, Isik F, Sayinsu K, Arun T, Aydemir B. Effect of blood and saliva contamination on shear bond strength of brackets bonded with 4 adhesives. *Am J Orthod Dentofacial Orthop.* 2007; 131: 238–242.
21. Oonsombat C; Bishara SE; Ajilouni R. The effect of blood contamination on the shear bond strength of orthodontic brackets with the use of a new self-etch primer. *Am J Orthod Dentofacial Orthop.* 2003; 123: 547–550.
22. Cunha T de MA, Behrens BA, Nascimento D, Retamoso LB, Lon LFS, Tanaka O, Guariza Filho O. Blood contamination effect on shear bond strength of an orthodontic hydrophilic resin. *J Appl Oral Sci.* 2010; 20: 89–93.
23. Cacciafesta V, Sfrondrini MF, De Angelis M, Scribante A, Klersy C. Effect of water and saliva contamination on shear bond strength of brackets bonded with conventional, hydrophilic, and selfetching primers. *Am J Orthod Dentofacial Orthop.* 2003; 123: 633–640.
24. Talic Nabeel F. Effect of fluoridated paste on the failure rate of precoated brackets bonded with self-etching primer: A prospective split-mouth study. *Am J Orthod Dentofacial Orthop.* 2011; 140: 527–530.
25. Reynolds IR. A review of direct orthodontic bonding. *Br J Orthod.* 1975; 2: 171–178.
26. International Organization of Standardization Technical Specification Report (ISO/TS 11405:2003).
27. Lill DJ, Lindauer. SJ, Tüfekçi E, Shroff B. Importance of pumice prophylaxis for bonding with self-etch primer. *Am J Orthod Dentofacial Orthop.* 2008; 133: 423–426.
28. Daub J, Berzins DW, Linn BJ, Bradley TG. Bond strength of direct and indirect bonded brackets after thermocycling. *Angle Orthod.* 2006; 76: 295–300.
29. Campoy MD, Plasencia E, Vicente A, Bravo La, Cibrián R. Effect of saliva contamination on bracket failure with self-etching primer: A prospective controlled clinical trial. *Am J Orthod Dentofacial Orthop.* 2010; 137: 679–683.