

[\[Print Version\]](#)

[\[PubMed Citation\]](#) [\[Related Articles in PubMed\]](#)

TABLE OF CONTENTS

[\[INTRODUCTION\]](#) [\[MATERIALS AND...\]](#) [\[RESULTS\]](#) [\[DISCUSSION\]](#) [\[CONCLUSIONS\]](#) [\[REFERENCES\]](#) [\[TABLES\]](#) [\[FIGURES\]](#)

The Angle Orthodontist: Vol. 73, No. 4, pp. 445–450.

In Vitro Investigation of Indirect Bonding with a Hydrophilic Primer

Arndt Klocke, Dr med dent, MS;^a Jianmin Shi, MSc;^b Bärbel Kahl-Nieke, Dr med dent, PhD;^c Ulrich Bismayer, Dr rer nat, PhD^d

ABSTRACT

The aim of this in vitro investigation was to evaluate bond strength for a custom base indirect bonding technique using a hydrophilic primer on moisture-contaminated tooth surfaces. Stainless steel brackets were bonded to 100 permanent bovine incisors using a light-cured custom base composite adhesive, a chemically cured sealant, and the hydrophilic primer Transbond MIP® (3M-Unitek, Monrovia, Calif). Five groups (A–E) of 20 teeth each were formed according to the time of contamination (before or after application of the primer) and the type of contaminant (distilled water or saliva): A, control group with no contamination; B, contamination with saliva before application of the primer; C, contamination with water before application of the primer; D, contamination with saliva before and after application of the primer; and E, contamination with water before and after application of the primer. Mean bond strength for the group without contamination (A) was 15.07 ± 4.14 MPa and was not significantly different from bond strengths for groups B (14.91 ± 3.99 MPa) and C (16.12 ± 3.67 MPa), in which contamination occurred before application of the hydrophilic primer. Average bond strength in group D was 11.92 ± 4.76 MPa. The lowest mean bond strength was measured for group E (9.85 ± 3.77 MPa) and was significantly lower than for groups A, B, and C. Contamination after primer application resulted in an increased risk of bond failure at clinically relevant levels of stress.

KEY WORDS: Indirect bonding, Hydrophilic primer, Bond strength, In vitro.

Accepted: December 2002. Submitted: November 2002

INTRODUCTION [Return to TOC](#)

Moisture contamination has been reported to be the most common reason for bond failure in clinical orthodontics.^{1,2} Saliva contamination of etched enamel seems to cause a significant decrease in bond strength between the resin and the enamel surface.^{3–5} This was shown by Hormati et al⁵ when composite resin was applied directly to a wet, saliva-contaminated surface. Silverstone et al⁶ reported that only with an exposure of less than one second to saliva could the contaminant be successfully removed by washing. Silverstone et al⁶ recommended that because any contamination occurring clinically will inevitably exist for one second or longer, the operator should not proceed with the bonding technique until the surface has been dried and reetched. Therefore, reetching is generally necessary for successful adhesion of the bracket, and it delays the bonding procedure considerably. Recently, two different hydrophilic bonding materials have been introduced: (1) moisture-insensitive or moisture-resistant primers, which contain water, acetone, or ethanol and tolerate the presence of moisture to a certain degree^{7–11} and (2) moisture-active adhesives, which are cyanoacrylate based; these adhesives not only tolerate but require the presence of moisture for the initiation of the polymerization process.^{12–15} In light of encouraging

results obtained using the hydrophilic materials, it has been suggested that the concept that saliva-contaminated enamel and dentin need to be reetched should be reconsidered.¹⁰

Indirect bonding techniques have been used for bonding in areas that are particularly susceptible to moisture contamination, eg, molar attachments or lingual brackets. Although it has been reported that improved moisture isolation may be rendered by the use of the transfer tray in indirect bonding,¹⁶ use of a moisture-resistant primer might be beneficial with this technique. However, until now, bond strength measurements with this primer have been limited to direct bonding techniques. Sonis¹¹ reported similar bond strength of orthodontic brackets applied to saliva-contaminated etched enamel and for uncontaminated enamel when the water-based primer Scotchbond Multipurpose® (3M-Unitek, Monrovia, Calif) was used. More recent studies tested the ethanol-containing hydrophilic primer Transbond MIP® (3M-Unitek). Littlewood et al⁹ demonstrated significantly lower bond strength with this primer in vitro. Although the median bond strength values were found to be promising, the Weibull analysis showed that brackets bonded with the hydrophilic primer seem to be at a higher risk of bond failure. These in vitro results were confirmed by a clinical study on 31 patients.¹⁷ An increased risk of bracket failure with the hydrophilic primer was found both for anterior and posterior teeth that were bonded, with an overall bond failure of 6.8% for the conventional adhesive and 18.8% for the hydrophilic primer. Webster et al¹⁸ reported lower bond strength measurements for Transbond MIP® for all contaminated surfaces when compared with uncontaminated surfaces. Hobson et al⁸ investigated the influence of both water and blood contamination and found significantly higher bond strength for the dry state compared to water or blood contamination. Despite these results, the authors concluded that the material was suitable for bonding under conditions of poor moisture control or blood contamination because all bond strengths measured were greater than the required clinical bond strength reported previously. Grandhi et al² investigated the Transbond MIP® primer in combination with a chemically cured and a light-cured adhesive. For the chemically cured adhesive, Grandhi et al² found a dramatic and highly significant drop in bond strength when enamel was contaminated with water or saliva, whereas bond strength with the light-cured adhesive was acceptable in the presence of a thin film of water or saliva. Therefore, Grandhi et al² concluded that the hydrophilic primer should only be used with light-activated composite adhesives.

No study so far has investigated the use of hydrophilic primers in indirect bonding. Therefore, the aim of this study was to evaluate bond strength for a custom base indirect bonding technique when using the hydrophilic primer on moisture-contaminated tooth surfaces.

MATERIALS AND METHODS [Return to TOC](#)

Bonding procedure

One hundred freshly extracted bovine permanent mandibular incisors were obtained from a local slaughterhouse and stored in 0.5% chloramine solution before the experiment. Teeth were randomly assigned to five groups of 20 specimens each. After cleaning the teeth with a brush and pumice-water slurry at slow speed, the teeth were embedded in chemically cured dental acrylic (Palavit G, Heraeus Kulzer, Wehrheim, Germany) in plastic cylinders to allow for standardized and secure placement during testing. Maxillary central incisor 0.018-inch-slot stainless steel mesh base brackets (Mini Mono, order no. 0712-0103, Forestadent, Pforzheim, Germany) were used throughout the study. The average surface area of the bracket base was 13.5 mm². The indirect bonding technique was performed in the following manner: an alginate impression was obtained of each specimen and poured in orthodontic stone. On the dry casts, the teeth were coated with diluted separating medium and allowed to dry for 24 hours. The bracket bases were cleaned with alcohol. The composite adhesive Transbond XT® (3M-Unitek) was applied to the bracket base. The brackets were pressed firmly onto the model. Excess composite was removed with a scaler. The adhesive was cured with a halogen-curing light (Polylux II, Kavo, Biberach, Germany) for two minutes. The output of the halogen light was measured at 800 mW/cm² before the experiment. The extended curing period was chosen to achieve complete polymerization of the adhesive on the plaster model.

After polymerization of the custom base adhesive, transfer trays were made from vinyl polysiloxane impression material (Silagum AV-Putty soft, DMG, Hamburg, Germany). After the transfer tray material had set, the specimens were soaked in warm water for 30 minutes. The transfer trays were removed from the models, and the composite adhesive on the bracket base was cleaned by sandblasting with 50 µm aluminum oxide.

Seven days after fabrication of the transfer trays, the second part of the bonding procedure was performed:¹⁹ the teeth were etched with 37% phosphoric acid gel (Ormco, Orange, Calif) for 30 seconds; rinsed thoroughly with water and air-water spray; and dried with compressed air for 20 seconds. The hydrophilic primer Transbond MIP® was used according to the manufacturer's recommendation: one liberal coat of the primer, covering the entire etched tooth surface, was applied. Tooth surfaces were contaminated with 0.1 ml of distilled water or fresh whole saliva from one volunteer who did not receive any medication. Fresh whole human saliva has been found to be suitable for testing saliva contamination.^{4,6,10,11,13} The following five groups were formed based on the contaminant (distilled water or saliva) and the timing of contamination (before or after application of the moisture-resistant primer):

- Group A (dry/dry): no contamination.
- Group B (saliva/dry): contamination with saliva before application of Transbond MIP® primer.
- Group C (water/dry): contamination with distilled water before application of the Transbond MIP® primer.

- Group D (saliva/saliva): contamination with saliva before and after application of Transbond MIP® primer.
- Group E (water/water): contamination with water before and after application of Transbond MIP® primer.

All groups were bonded with Sondhi Rapid Set® adhesive (3M-Unitek) according to the manufacturer's recommendations. After bonding of the sealant was completed, the transfer trays were removed. In case of bracket failure on removal of the tray, the adhesive was removed from the tooth surface with a finishing bur, and the custom base of the bracket was cleaned with a scaler and sandblasted, and the bonding procedure was repeated. The specimens were stored in distilled water for 24 hours.

Debonding procedure

The brackets were debonded with a Zwicky Z2.5 universal testing machine (Zwick, Ulm, Germany) at a crosshead speed of one mm/min.^{8,20} The plastic cylinders with the embedded teeth and brackets were mounted on a joint and were aligned in the testing apparatus to ensure consistency for the point of force application and direction of the debonding force for all specimens. A stainless steel wire loop (0.020-inch diameter) was engaged under the occlusal bracket wings to produce a shear-peel force parallel to the bracket base in an occlusogingival direction. The load at failure was recorded.

For each specimen, the substrate surface was examined with an optical stereomicroscope (magnification 10×), and an adhesive remnant index (ARI) was determined,²¹ which is as follows:



- 0, no adhesive left on the tooth.
- 1, less than half of the adhesive left on the tooth.
- 2, more than half of the adhesive left on the tooth.
- 3, all the adhesive left on the tooth, with distinct impression of the bracket mesh.


The ARI score was assessed by the same operator.


Statistical analysis

To calculate shear bond strength, the debonding forces in Newton were converted to stress values in megapascals by taking into account the surface area of the bracket base. Bond strengths of the different groups were compared by one-way analysis of variance (ANOVA), with post hoc Tukey tests ($P < .05$). A Weibull analysis was performed; the Weibull modulus, characteristic bond strength, correlation coefficient, and the level of stress at 10% probability of failure were calculated. Kruskal-Wallis and Mann-Whitney nonparametric tests were used to determine whether there were any significant differences in the ordinal ARI values ($P < .05$).^{22,23}

RESULTS [Return to TOC](#)

The mean shear bond strengths, standard deviations, and parameters of the Weibull analysis (modulus, correlation coefficient, characteristic bond strength, stress at 10% probability of failure) are given in [Table 1](#) . [Figure 1](#)  shows the Weibull distribution plots of the probability of failure at a certain shear stress level for the different groups.

In group B, bond failure occurred in one specimen when the transfer tray was removed from the cast. The bonding procedure was repeated for this specimen. ANOVA showed that there were significant differences in shear bond strength among the groups investigated ($F = 8.118$, $P < .001$). The results of the post hoc Tukey tests demonstrated significant differences among the groups (see [Table 1](#) ). Although groups A, B, and C were not significantly different from each other, bond strength was found to be significantly lower for group D. Mean bond strength for group E was significantly lower than for group C.

No enamel fractures were found in any of the specimens. Median, mean, standard deviation, and range of the ARI scores are given in [Table 2](#) . The Kruskal-Wallis test indicated that there were significant differences among the groups ($\chi^2 = 11.29$, $P < .05$). The Mann-Whitney tests showed that group D had significantly lower ARI measurements than did group A ($P < .01$), whereas the ARI scores for all other group comparisons were not found to be significantly different from each other.

DISCUSSION [Return to TOC](#)

One of the problems in the investigation of the hydrophilic primer is that the effectiveness of the material may vary with the degree of

moisture contamination.² There seems to be a limit as to how much of a wet field is acceptable and excessive surface moisture can result in a decrease in bond strength.²⁴ Furthermore, the manufacturer recommends applying a “liberal coat of the primer.” This is different from recommendations for conventional primers and may introduce an additional variable; the definition of “liberal” is certainly subjective. The manufacturer's instructions for the use of the moisture-insensitive primer explain that although it is not critical to have moisture contamination after etching the tooth surface and before application, the application of the primer needs to be repeated if contamination occurs after the primer has been used. However, this will not necessarily be possible when bonding is performed in areas that are difficult to isolate; it may be infeasible to avoid contamination after the primer has been applied and before placing the transfer tray on the tooth, and saliva contamination may go unnoticed. Therefore, two different scenarios of contamination seem relevant in a clinical setting and were simulated in this study: (1) contamination occurs before the primer is applied and (2) contamination occurs both before and after application of the primer. Although the results showed a significant decrease in bond strength for the latter situation compared with bonding to uncontaminated etched enamel, mean bond strength measurements of 9.85 MPa (group D) and 11.92 MPa (group E) were found.

Bovine permanent mandibular incisors were used in this study. Bond strength measurements for bovine teeth have been found to be equal to, slightly lower, or lower than for human teeth.^{25–28} In general, interstudy comparison of bond strength measurements is complicated by a variety of materials and methods that have been used in bond strength studies;²⁹ these include variations in tooth type, storage conditions, method of debonding, analysis of the results, and the selection of products for comparison.³⁰

The rationale for applying a Weibull analysis for bond strength testing was outlined by Hobson et al.⁸ The Weibull analysis gives information about the probability of bracket failure in the worst scenario, thus the most detrimental type of moisture contamination, and may allow for comparison with bond failures reported in previous clinical studies. When comparing results of in vitro and in vivo studies on the hydrophilic primer, Littlewood et al.^{9,17} emphasized that the lower values of the bond strength distribution govern the likelihood of clinical failure. Littlewood et al.^{9,17} suggested using the 5% chance of failure as a more appropriate level to assess bond strength. According to these authors, the bond strength of a material with a 5% chance of failure should be at least 5.4 MPa. In this study, this requirement was met only by groups A, B, and C. Groups D and E showed lower bond strength at the 5% probability of failure. Hobson et al.⁸ proposed the calculation of probability of failure at the clinically sufficient bond strength level of eight MPa as recommended by Reynolds.³¹ When using the moisture-resistant primer in direct bonding techniques, Hobson et al.⁸ found a 14% chance of bond failure for the worst case scenario (blood contamination). In our study, groups A, B, and C showed a lower probability of failure at this level of stress. However, groups D and E were characterized by failure probabilities of 33% and 28%, respectively, which are higher than those that are clinically acceptable. This demonstrates that despite mean bond strength measurements of 9.85 and 11.92 MPa in these two groups, there is an increased risk of bond failure when contamination occurs after application of the hydrophilic primer. For group D, ARI scores were significantly lower than for the control group A, indicating that contamination with saliva after application of the hydrophilic primer resulted in a shift of the weak link of the bond toward the enamel-adhesive interface.

In a recent study, Grandhi et al.² found higher bond strength when the hydrophilic primer was used in combination with a light-cured adhesive compared to chemically cured adhesive. Grandhi et al.² speculated that when used in a wet field, the primer becomes diluted and that the hydrophobic nature of the chemically cured adhesive repels the primer, resulting in inadequate bond strength. According to these authors, there is potentially no mechanism for hardening of the hydrophilic primer because there is no application of visible light. The chemically cured composite adhesive used by Grandhi et al.² in their study was Concise® (3M-Unitex, Monrovia, Calif.). It was hypothesized that the hydrophobic nature of Concise® adhesive repels the primer. The present study used Sondhi Rapid Set® adhesive. This material was developed specifically for indirect bonding purposes. It is also chemically cured but is a completely different adhesive system in terms of viscosity and setting time compared with Concise®. Our results indicate that this adhesive seems to be compatible with the hydrophilic primer and results in adequate bond strength in a dry and a wet field. This demonstrates that the hydrophilic primer does not necessarily require a light-cured adhesive to allow for adequate polymerization. Therefore, the hydrophilic primer can be recommended for use with the indirect bonding technique used in this study.

CONCLUSIONS [Return to TOC](#)

1. Bond strength for the custom base indirect bonding technique with the hydrophilic primer was not significantly different in groups without contamination and with water or saliva contamination before application of the primer.
2. Moisture contamination after application of the hydrophilic primer resulted in significantly lower bond strength measurements compared with bond strength for uncontaminated enamel. Although average bond strength values of 9.85 ± 3.77 MPa (group D, saliva contamination) and 11.92 ± 4.76 MPa (group E, water contamination) were measured for these groups, the Weibull analysis indicated a higher risk of bond failure at clinically relevant levels of stress.

REFERENCES [Return to TOC](#)

1. Zachrisson BJ. A posttreatment evaluation of direct bonding in orthodontics. *Am J Orthod.* 1977; 71:173–189. [[PubMed Citation](#)]
2. Grandhi RK, Combe EC, Speidel TM. Shear bond strength of stainless steel orthodontic brackets with a moisture-insensitive primer. *Am*

J Orthod Dentofacial Orthop. 2001; 119:251–255. [[PubMed Citation](#)]

3. Gwinnett AJ. Moist versus dry dentin: its effect on shear bond strength. *Am J Dent.* 1992; 5:127–129. [[PubMed Citation](#)]

4. Thomson JL, Main C, Gillespie FC, Stephen KW. The effect of salivary contamination on fissure sealant-enamel bond strength. *J Oral Rehabil.* 1981; 8:11–18. [[PubMed Citation](#)]

5. Hormati AA, Fuller JL, Denehy GE. Effects of contamination and mechanical disturbance on the quality of acid etched enamel. *J Am Dent Assoc.* 1980; 100:34–38. [[PubMed Citation](#)]

6. Silverstone LM, Hicks MJ, Featherstone MJ. Oral fluid contamination of etched enamel surfaces: an SEM study. *JADA.* 1985; 110:329–332. [[PubMed Citation](#)]

7. Swift EJ, Perdigão J, Heymann HO. Enamel bond strengths of “one-bottle” adhesives. *Pediatr Dent.* 1998; 20:259–262.

8. Hobson RS, Ledvinka J, Meechan JG. The effect of moisture and blood contamination on bond strength of a new orthodontic bonding adhesive. *Am J Orthod Dentofacial Orthop.* 2001; 120:54–57. [[PubMed Citation](#)]

9. Littlewood SJ, Mitchell L, Greenwood DC, Bubb NL, Wood DJ. Investigation of a hydrophilic primer for orthodontic bonding: an in vitro study. *J Orthod.* 2000; 27:181–186.

10. El-Kalla IH, García-Godoy F. Saliva contamination and bond strength of single-bottle adhesives to enamel and dentin. *Am J Dent.* 1997; 10:83–87. [[PubMed Citation](#)]

11. Sonis AL. Effect of a new bonding agent on bond strength to saliva-contaminated enamel. *J Clin Orthod.* 1994; 28:93–94. [[PubMed Citation](#)]

12. Bishara SE, Laffoon JF, VonWald L, Warren J. Effect of time on the shear bond strength of cyanoacrylate and composite orthodontic adhesives. *Am J Orthod Dentofacial Orthop.* 2002; 121:297–300. [[PubMed Citation](#)]

13. Eliades T, Katsavrias E, Eliades G. Moisture-insensitive adhesives: reactivity with water and bond strength to wet and saliva-contaminated enamel. *Eur J Orthod.* 2002; 24:35–42. [[PubMed Citation](#)]

14. Howells DJ, Jones P. In vitro evaluation of a cyanoacrylate bonding agent. *Br J Orthod.* 1989; 16:75–78. [[PubMed Citation](#)]

15. Örtendahl TW, Örtengren U. A new orthodontic bonding adhesive. *J Clin Orthod.* 2000; 34:50–54.

16. Read MJF, O'Brien KD. A clinical trial of an indirect bonding technique with a visible light-cured adhesive. *Am J Orthod Dentofacial Orthop.* 1990; 98:259–262. [[PubMed Citation](#)]

17. Littlewood SJ, Mitchell L, Greenwood DC. A randomized controlled trial to investigate brackets bonded with a hydrophilic primer. *J Orthod.* 2001; 28:301–305.

18. Webster MJ, Nanda RS, Duncanson MG, Khajotia SS, Sinha PK. The effect of saliva on shear bond strengths of hydrophilic bonding systems. *Am J Orthod Dentofacial Orthop.* 2001; 119:54–58. [[PubMed Citation](#)]

19. Shiau JY, Rasmussen ST, Phelps AE, Enlow DH, Wolf GR. Bond strength of aged composites found in brackets placed by an indirect technique. *Angle Orthod.* 1993; 63:213–220. [[PubMed Citation](#)]

20. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. *Am J Orthod Dentofacial Orthop.* 1998; 114:514–519. [[PubMed Citation](#)]

21. Artun J, Bergland S. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. *Am J Orthod.* 1984; 85:333–340. [[PubMed Citation](#)]

22. Bulman JS, Osborn JF. Significance tests. Part 3. *Br Dent J.* 1989; 166:261–264. [[PubMed Citation](#)]

23. Sheats RD, Pankratz VS. Common statistical tests. *Semin Orthod.* 2002; 8:77–86.

24. Tay FR, Gwinnett AJ, Wei SHY. The overwet phenomenon: an optical, micromorphological study of surface moisture in the acid-conditioned, resin-dentin interface. *Am J Dent.* 1996; 9:43–48. [[PubMed Citation](#)]

25. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. *Am J Orthod Dentofacial Orthop.* 1998; 114:514–519. [[PubMed Citation](#)]

26. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitutes in the adhesion test. *J Dent Res.* 1983; 62:1076–1081. [\[PubMed Citation\]](#)

27. Spitzer D, Ten Bosch JJ. The total luminescence of bovine and human dental enamel. *Calcif Tissue Res.* 1976; 20:201–208.

28. Smith HZ, Casko JS, Leinfelder KF, Utley JD. Comparison of orthodontic bracket bond strengths: human versus bovine enamel [abstract 367]. *J Dent Res.* 1976; 55:B153

29. Fox NA, McCabe JF, Buckley JG. A critique of bond strength testing in orthodontics. *Br J Orthod.* 1994; 21:33–43. [\[PubMed Citation\]](#)

30. Eliades T, Brantley WA. The inappropriateness of conventional orthodontic bond strength assessment protocols. *Eur J Orthod.* 2000; 22:13–23. [\[PubMed Citation\]](#)

31. Reynolds IR. A review of direct orthodontic bonding. *Br J Orthod.* 1975; 2:171–178.

TABLES
 [Return to TOC](#)

TABLE 1. Shear Bond Strength (Mean, Standard Deviation [SD]) and Weibull Parameters

Group	Mean (MPa)	SD (MPa)	Group Differences ^a	Weibull Modulus	Correlation Coefficient	Characteristic Bond Strength (MPa)	Shear Stress at 10% Probability of Failure (MPa)
A (dry/dry)	15.07	4.14	a,b	2.57	0.881	17.60	7.32
B (saliva/dry)	14.91	3.99	a,b	3.52	0.966	16.67	8.79
C (water/dry)	16.12	3.67	b	3.22	0.876	18.37	9.15
D (saliva/saliva)	9.85	3.77	c	2.85	0.977	11.07	5.03
E (water/water)	11.92	4.76	a,c	2.02	0.959	13.97	4.58

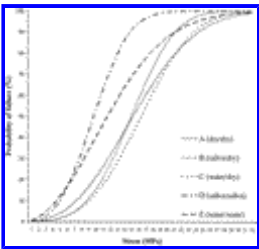
^a Groups with the same letters are not significantly different from each other (Tukey, P < .05).

TABLE 2. Frequency Distribution of Adhesive Remnant Index (ARI) Scores of the Groups Tested^a

Group	ARI scores							
	0	1	2	3	Median	Mean	SD	Range
A (dry/dry)	—	9	11	—	2.00	1.55	0.51	1–2
B (saliva/dry)	—	14	6	—	1.00	1.30	0.47	1–2
C (water/dry)	—	12	8	—	1.00	1.40	0.50	1–2
D (saliva/saliva)	1	17	2	—	1.00	1.05	0.39	0–2
E (water/water)	1	14	5	—	1.00	0.20	0.52	0–2

^a SD indicates standard deviation. ARI: 0, no adhesive left on the tooth; 1, less than half of the adhesive left on the tooth; 2, more than half of the adhesive left on the tooth; 3, all the adhesive left on the tooth, with distinct impression of the bracket mesh.

FIGURES
 [Return to TOC](#)



Click on thumbnail for full-sized image.

FIGURE 1. Weibull distribution plots. Groups—A (dry/dry): no contamination; B (saliva/dry): contamination with saliva before application of the primer; C (water/dry): contamination with distilled water before application of the primer; D (saliva/saliva): contamination with saliva

^aAssociate Professor, Department of Orthodontics, College of Dentistry, University of Hamburg, Germany

^bPhD student, Department of Earth Sciences, Institute of Mineralogy, University of Hamburg, Germany

^cProfessor and Chair, Department of Orthodontics, University of Hamburg, Germany

^dProfessor and Head, Department of Earth Sciences, Institute of Mineralogy, University of Hamburg, Germany

^aCorresponding author: Arndt Klocke, Dr med dent, MS, Department of Orthodontics, Kieferorthopaedie, ZMK-Klinik, Pav. O 53, U.K.E., Martinistr. 52, 20246 Hamburg, Germany (E-mail: klocke@uke.uni-hamburg.de)