The Influence of Storage Time and Cutting Speed on Microtensile Bond Strength

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Purpose: The aim of this study was to verify the influence of the storage time and the cutting speed during specimen preparation on the bond strength of a single-bottle adhesive to dentin.

Materials and Methods: A flat dentin surface was exposed in 36 human third molars. The adhesive system (Single Bond) was applied according to the manufacturer's instructions, and composite resin crowns (Z250) were constructed incrementally. Specimens were stored for 10 min, 24 h, or 1 week in distilled water at 37°C before being longitudinally sectioned in both the "x" and "y" directions at different cutting speeds (0.5, 1.6, and 2.6 m/s) to obtain sticks with a cross-sectional area of approximately 0.8 mm^2. The specimens were tested in a tensile load machine (0.5 mm/min) and the fracture mode analyzed. A two-way ANOVA with storage time (3 levels) and cutting speed (3 levels) as factors was used to compare the mean microtensile bond strengths.

Results: Highly significant main effects and interaction ($p < 0.0001$) were detected. The highest mean bond strength was obtained with a storage time of 1 week and cutting speed of 2.6 m/s. The lowest mean was found when the specimens were prepared immediately after composite resin placement and sliced at 0.5 m/s.

Conclusion: Both the storage time and the cutting speed may affect the bond strength results. Therefore, these variables must be controlled in microtensile bond strength tests.

Key words: microtensile test, cutting speed, resin-dentin bond strength.

J Adhes Dent 2004; 6: 7–11. *Submitted for publication: 20.02.03; accepted for publication: 12.05.03.*

In order to determine the properties of bonding systems, many researchers have carried out a variety of bond strength evaluations. Recently, Al-Salehi and Burke[1] reviewed test methods and variables used in 50 published investigations of bond strength, finding that shear and tensile tests are the most commonly used. However, several drawbacks have been reported about these methods, mainly those related to nonuniform stress distribution.[21,22]

The microtensile test was primarily proposed to evaluate the bond strength between adhesives and very small regions of dental tissue; the conclusion was reached that the bonded interface of small specimens had a better stress distribution during test loading.[21,22] This test involves bonding the adhesive resin to dentin, which is then covered with a composite resin. After curing and storage, the specimen is vertically sectioned into multiple serial slabs and trimmed to reduce the cross-sectional area to about 0.8 mm^2 before testing.[29] Although the original version is still being used, many other approaches have been introduced. One of them was the so-called nontrimming microtensile test, in which the composite resin build-up is sectioned vertically and perpendicular to the adhesive interface into 5 or 6 slabs; however, the blade does not pass entirely through the dentin. Then, after rotating the specimen 90 degrees, another 5 to 6 sections are made, resulting in 25 to 30 sticks in molar teeth.[31]

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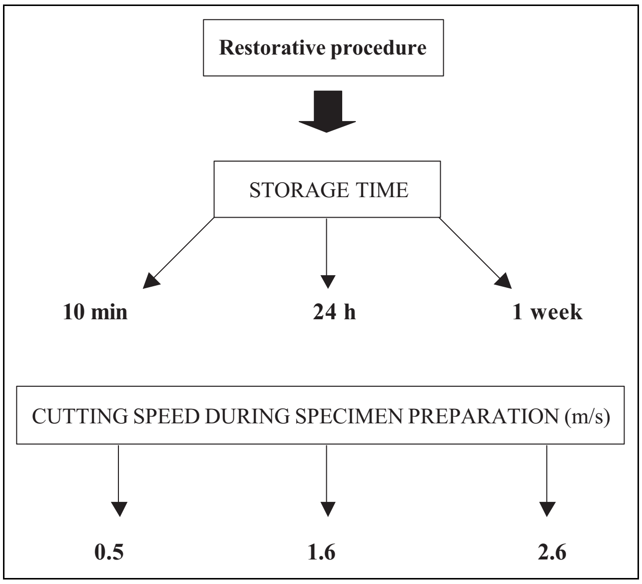

Fig 1 Experimental design of the study.

The number of cohesive failures was significantly reduced with the microtensile test.[29] This test has been used to measure differences in regional bond strength across occlusal dentin,[24,31] down the external surface of teeth from crown through roots,[34,36] down the internal surface of root canals from cervical to apical thirds,[15] and to compare normal vs caries-affected occlusal dentin[17,18,37] and sclerotic cervical dentin,[35,36] etc.

Despite the numerous possibilities of evaluating resin-dentin bond behavior with the microtensile testing method, there is concern about its reproducibility, as a closer analysis of those studies reveals different bond strengths even when the same adhesive system was tested under quite similar experimental conditions.[2,8,18,26] This may be attributed to several inherent variables in the bonding procedure, such as adhesive composition, adhesion technique, operator's ability, and certain intrinsic properties of dental tissues; however, methodological differences may also play an important role in the disparate published results.

Factors such as shape of the specimen, size of bonded interface, crosshead speed during testing, specimen grinding, storage protocols, and the cutting speed during specimen preparation also vary in the published studies.[2,8,9,12,13,19,23,24,28] Although several authors have reported that the specimens were sectioned with a low-speed diamond saw,[7,10,11,20] none of them indicated the actual cutting speed used for this purpose. The need for some form of standardized adhesion test has been widely recognized, but few efforts have been made towards this end.[33]

Aside from long-term studies, bond strength studies are commonly performed 24 h after bonding procedures. Time could be saved if specimens were cut immediately after the restorative procedure with a high-speed diamond saw.

To date, there has been no systematic evaluation of the variables mentioned above, although some studies have warned that some methodological differences can lead to different bond strength data.[9,12,13]

The aim of this study was therefore to verify the influence of the storage time and the cutting speed during specimen preparation on the bond strength values of a single-bottle adhesive to dentin. The null hypothesis was that there is no influence of storage periods or cutting speeds on resin-dentin bond strengths.

MATERIALS AND METHODS

Tooth Preparation

The 36 teeth used in this study were obtained according to protocols properly approved by the institutional review board and with informed consent of the donors. The teeth were stored in 0.5% chloramine[5] and used within 6 months of extraction. The sample was divided into 9 experimental groups, assigning 4 teeth to each condition, as shown in Fig 1.

A flat dentin surface was exposed after grinding the occlusal enamel. Then the dentin surfaces were polished on wet 600-grit silicon carbide paper for 60 s to create a standardized smear layer. An effort was made to achieve superficial dentin surfaces.

Adhesive and Restorative Procedures

The adhesive system (Single Bond, 3M ESPE, St Paul, MN, USA) was applied according to the manufacturer's instructions (Table 1) and activated using a VIP light source with an output of 600 mW/cm^2 (Bisco, Schaumburg, IL, USA). Composite resin "crowns" (Z250, 3M ESPE) were constructed using increments of 1 mm, each light cured for 30 s. The composition, application mode, and batch numbers are detailed in Table 1.

Then the specimens were stored in distilled water at 37°C for 10 min, 24 h, or 1 week after the composite resin placement. After these respective storage times, the specimens were sectioned in the "x" and "y" directions by means of a diamond saw in a Labcut 1010 machine (Extec, Enfield, CT, USA) at 100, 300, or 500 revolutions per minute in order to obtain sticks with a rectangular cross-sectional area of approximately 0.8 mm^2. The actual angular velocity can be calculated by the following expression: $V = 2\pi rf$, in which $2\pi r$ is the complete displacement of a point situated in the periphery of the saw (dependent upon the radius of the saw) and f is the frequency of that displacement (eg, in rpm, as expressed above). Considering that the radius of the saw used was approximately 0.05 m (5 cm), the cutting speeds evaluated were ca 0.5, 1.6, and 2.6 m/s, resp. In order to verify the dentin depth where the bonding procedures were performed, the length of the remaining dentin from bonded surface to nearest pulp horn was measured using a digital micrometer (Absolute Digimatic, Mitutoyo, Tokyo, Japan), as was the cross-sectional area of each specimen.

Table 1 Composition, adhesive procedure, and batch number

Adhesive	Composition	Adhesive procedure	Batch number
Single Bond (3M ESPE, St Paul, MN, USA)	1. Scotchbond: 35% phosphoric acid gel 2. Adhesive: bis-GMA, HEMA, dimethacrylates, polyalquenoic acid copolymer, initiators, water, ethanol	1. Acid etch (15 s) 2. Rinse (15 s) 3. Air dry (30 s) 4. Dentin rewetted with 1.5 µl of water 5. Application of two coats of adhesive systems, brushed for 10 s each 6. Air dry for 10 s at 20 cm 7. Light activation (10 s with 600 mW/cm^2).	9CX

Table 2 Number of sticks showing adhesive/mixed fracture pattern (A/M) and premature debonding (D) for each experimental group

cutting speed (m/s)	10 min		24 h		1 week	
	A/M	D	A/M	D	A/M	D
0.5	60	6	81	2	74	4
1.6	75	7	61	4	67	2
2.6	53	7	67	2	71	4

Table 3 Mean bond strengths and standard deviation (MPa)

cutting speed (m/s)	10 min	24 h	1 week
0.5	33.9 ± 12.6 ^{bc}	33.9 ± 14.7 ^{b}	34.0 ± 16.6 ^{bc}
1.6	38.2 ± 13.1 ^{b}	46.2 ± 15.9 ^{ab}	35.3 ± 11.9 ^{b}
2.6	35.9 ± 14.8 ^{b}	42.5 ± 15.3 ^{ab}	48.9 ± 18.9 ^{a}

Same letters indicate no significant differences (α = 0.05).

Microtensile Test

The “sticks” were attached to a Geraldeli’s device,[23] which assured uniaxial movement, with cyanoacrylate resin (Zapit, DVA, Corona, CA, USA) and subjected to a tensile force in a universal testing machine (Kratos Dinamometros, São Paulo, SP, Brazil) at a crosshead speed of 0.5 mm/min. The bond failure modes were evaluated at 400X magnification with a stereomicroscope (HMV-2, Shimadzu, Tokyo, Japan) and classified as cohesive (failure within dentin or composite resin, C), adhesive (failure between dentin/composite resin), or mixed (combination of adhesive and cohesive failures, A/M). Only the sticks that showed adhesive or mixed fracture pattern were used for the calculation of the mean bond strengths of each experimental group.

Statistical Analysis

The data were analyzed by two-way analysis of variance (ANOVA) at a confidence level of 95%. The storage time and cutting speed were the two main factors. In addition, the interaction between the factors was also evaluated. Post hoc multiple comparisons were performed using Tukey’s test set at a level of 95%.

RESULTS

The remaining dentin thickness for all specimens ranged from 2.0 to 2.7 mm, indicating that the interfaces were located in mid-depth dentin.[3] The mean cross-sectional areas ranged from 0.76 to 0.84 mm^2 and no difference among the treatment groups was detected ($p = 0.82$).

No cohesive failure was found among the sticks tested. The number of sticks showing adhesive or mixed fractures are shown in Table 2, as well as the number of sticks that did not survive test preparation. As the number of specimens that debonded during test preparation was small and equally distributed among experimental groups (data not shown), they were not considered in the statistical analysis. The means and standard deviations of the bond strength values are shown in Table 3. The two-way ANOVA detected statistical differences for the main factors storage time ($p = 0.001$) and cutting speed ($p = 0.006$), and for their interaction ($p = 0.001$).

The highest mean bond strength was obtained in specimens stored for 1 week and sliced at a speed of 2.6 m/s. The lowest mean bond strength was obtained for specimens cut immediately after the restorative procedure and at the lowest cutting speed.

DISCUSSION

The importance of high tensile bond strengths of resin materials to enamel and dentin in creating well-sealed and long-lasting restorations has often been stated.[4,10,11,19] High bond strength values are desirable in order to withstand the stress that arises from polymerization shrinkage and to avoid debonding due to masticatory forces. In order to overcome the latter, early bond strengths are considered important, since most shrinkage and polymerization stress occurs immediately after activation of light-cured composites.[6,30] Burrow et al[3] have addressed the difference in bond strength values when the specimens were tested under tensile forces at 1 min, 10 min, and 24 h after activation, finding higher bond strengths when specimens were measured at 24 h. Muñoz-Viveros et al[16] testing several adhesive systems at 30 min and 24 h under shear forces reached similar conclusions for most adhesive systems, although One-Step (Bisco) and Prime & Bond (Dentsply) did not show any statistical differences regarding the tested periods.

The highest bond strength value in the present study was obtained when the specimens were tested after 1 week. The increase in bond strength over 1 week was not surprising, since previous work on resin composites has shown that the polymerization continues for at least 24 h after initiation.[14,27] Despite conflicting results, this variable must be standardized in laboratory studies in order to avoid variations among experimental groups. For instance, Bouillaguet et al[2] tested the bond strength values of different adhesive systems to bovine dentin, finding lower bond strength values than found in other studies. The authors attributed this difference to the fact that the specimens were obtained immediately after the composite resin placement. Although a storage time of 24 h is usually reported in most of the studies, storage times of 48 h,[24,28] 3 days,[23] 1 week,[19] and 2 weeks[8] are also mentioned in the literature.

After the bonding and restorative procedures, the area of the specimen in a microtensile test is determined by slicing the tooth/restoration interface in the "x" and "y" directions in order to obtain sticks for testing. This procedure likely causes slight eccentric movements of the diamond saw, depending on the set cutting speed. This phenomenon, associated with the friction between the specimen and the saw, generates stresses that can be transferred to the adhesive interface, causing in turn premature debonding or lower bond strengths of some sticks.

To a certain extent, this concern has been addressed in most of the recent articles. They have attempted to report either the number of specimens that survived preparation[11] or the opposite, ie, the number of debonded specimens.[4,19,25,32] In the present study, as the bonding protocol was carefully executed and no inherent variables of bonding procedure were evaluated, few sticks failed before testing and no difference was detected regarding this variable. On the other hand, the bond strengths varied among the experimental groups, showing a possible effect of the factors studied.

Undoubtedly, a high incidence of debonded specimens during preparation suggests a greater fragility of the bond, which makes the specimens more susceptible to early failure. The lowest bond strength values obtained in a study can indirectly indicate the amount of stress that can initiate or propagate critically sized defects in the specimen during its preparation. For instance, Bouillaguet et al[2] indicated that most specimens which debonded during preparation could have had bond strengths as high as 13 MPa, although Pashley et al[21] maintain that such prematurely debonded specimens would have values of under 4 MPa. Differences in specimen preparation as well as other unknown and known factors (such storage time before slicing) could have accounted for the difference between the two studies.

A bond strength index can be created, taking into consideration the relative contribution of all types of bond failures and even prematurely debonded specimens to yield a strength index for each individual tooth employed in microtensile studies.[26] The final bond strength index for each experimental variable can be then calculated as an average of all teeth used in a particular experimental group.

We expected that all groups would present higher bond strengths under higher cutting speeds, based on the supposition that a reduced cutting time would diminish the chance of amplifying any flaws previously present in the specimens or initiating new ones. In addition, increasing cutting speed probably reduces the magnitude of the disk's oscillatory movements, consequently reducing stress-related damage induced during the slicing process. However, this hypothesis was not completely confirmed in the present study, since not all groups showed the same trend. A strong interaction was found between the storage time and cutting speed, which made the evaluation of a sole factor difficult.

Another factor not yet addressed is the diameter of the diamond saw disk. We believe that this variable also influences the magnitude of the transferred stresses. The larger the disk diameter, the higher is the tangential speed. Thus, the periphery of larger disks moves a greater distance than that of the smaller ones at the same number of rpms; consequently, they cut faster than smaller disks. On the other hand, the greater diameter disks also have more eccentric movements, which may cause more stress on the interface. Further studies must be conducted in order to elucidate this matter.

An understanding of the conditions which impact the test protocol is imperative when conducting in vitro testing. In cases where only a short time has elapsed between placing a composite resin restoration and testing, a high cutting speed should be chosen in order to minimize detrimental effects of incomplete polymerization of those specimens. Further studies must be conducted in order to prove the hypothesis stated in this study.

CONCLUSION

The results of this study show that storage time and/or the cutting speed may affect the microtensile bond strength values, and these variables must therefore be standardized in microtensile bond strength tests.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Dental Materials, School of Dentistry – University of São Paulo (USP) and to the School of Dentistry – University of Oeste of Santa Catarina – Joaçaba – SC, Brazil. This research was partially support by CNPq grant 350085/2003-0.

REFERENCES

1. Al-Salehi SK, Burke FJT. Methods used in dentin bonding tests: an analysis of 50 investigations on bond strength. Quintessence Int 1997;28: 717-723.
2. Bouillaguet S, Ciucchi B, Jacoby T, Wataha JC, Pashley DH. Bonding characteristics to dentin walls of class II cavities, in vitro. Dent Mater 2001;17: 316-321.
3. Burrow MF, Tagami J, Negishi T, Nikaido T, Hosoda H. Early tensile bond strengths of several enamel and dentin bonding systems. J Dent Res 1994;73:522-528.
4. Carrilho MRO, Reis A, Loguercio AD, Rodrigues Filho LE. Resistência de união à dentina de quatro sistemas adesivos. Pesqui Odontol Brasil 2002; 16:251-256.
5. De Wald JP. The use of extracted teeth for in vitro bonding studies: a review of infection control considerations. Dent Mater 1997;13:74-81.
6. Feilzer AJ, De Gee AJ, Davidson CL. Quantitative determination of stress reduction by flow in composite restoration. Dent Mater 1990;6:167-171.
7. Foxton RM, Pereira PNR, Nakajima M, Tagami J, Pashley DH. Long-term durability of the dual-cure resin cement/silicon oxide ceramic bond. J Adhes Dent 2002;4:125-136.
8. Guzman-Ruiz S, Armstrong SR, Cobb DS, Vargas MA. Association between microtensile bond strength and leakage in the indirect resin composite/dentine adhesively bonded joint. J Dent 2001;29:145-153.
9. Houston RA, Chan DCN, Pashley DH, Armstrong SR. Comparison of microtensile strength using Ciucchi and Dirck's devices [abstract 3842]. J Dent Res 2002;81:A-470.
10. Ibarra G, Vargas MA, Armstrong SR, Cobb DS. Microtensile bond strength of self-etching adhesives to ground and unground enamel. J Adhes Dent 2002;4:115-124.
11. Inoue H, Inoue S, Uno S, Takahashi A, Koase K, Sano H. Microtensile bond strength of two single-step adhesive systems to bur-prepared dentin. J Adhes Dent 2001;3:129-136.
12. Itou K, Tay FR, Torii Y, Agee K, Ceballos L, Carvalho RM, Yoshiyama M, Pashley DH. Effect of testing speed and gripping on micro-tensile bond strength [abstract 3361]. J Dent Res 2002;81:A-415.
13. Larson WK, Geraldeli S, Perdigão J, Hodges JS. Sources of variation in dentin micro-tensile bond strength testing [abstract 407]. J Dent Res 2002;81:A-76.
14. Leung RL, Adishian SR, Fan PL. Postirradiation comparison of photoactivated composite resin. J Prosthet Dent 1985;54:645-649.
15. Mannocci F, Sherriff M, Ferrari M, Watson TF. Microtensile bond strength and confocal microscopy of dental adhesives bonded to root canal dentin. Am J Dent 2001;14:200-204.
16. Munoz-Viveros CA, Dun JR, Liu P, Sy-Munoz J, Kajihara S. Early bond strength of six bonding systems [abstract 1391]. J Dent Res 1997;76: 187.
17. Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu T, Pashley DH. Tensile bond strength and SEM evaluation of caries-afffected dentin using dentin adhesives. J Dent Res 1995;4:1679-1688.
18. Nakajima M, Sano H, Zheng L, Tagami J, Pashley DH. Effect of moist vs. dry bonding to normal vs. caries-afffected dentin with Scotchbond Multi-Purpose Plus. J Dent Res 1999;78:1298-1303.
19. Nikaido T, Kunzelmann K-H, Ogata M, Harada N, Yamaguchi S, Cox CF, Hickel R, Tagami J. The in vitro dentin bond strengths of two adhesive systems in class I cavities of human molars. J Adhes Dent 2002;4:31-40.
20. Nunes MF, Swift Jr EJ, Perdigão J. Effects of demineralization depth on microtensile bond strength to human dentin. J Adhes Dent 2001;3: 137-144.
21. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay FR. The microtensile bond test: a review. J Adhes Dent 1999;1:299-309.
22. Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: A review. Dent Mater 1995;11:117-125.
23. Perdigão J, Geraldeli S, Carmo ARP, Dutra HR. In vivo influence of residual moisture on microtensile bond strengths of one-bottle adhesives. J Esthet Rest Dent 2002;14:31-38.
24. Phrukkanon S, Burrow MF, Tyas MJ. The effect of dentin location and tubule orientation on the bond strength between resin and dentin. J Dent 1999; 27:265-274.
25. Reis A, Loguercio AD, Grande RHM. Preliminary study of the moisture spectrum for different solvent-based adhesive systems [abstract 1843]. J Dent Res 2002;81:A-241.
26. Reis A, Loguercio AD, Azevedo CLN, Carvalho RM, Singer JM, Grande RHM. Moisture spectrum of demineralized dentin for adhesive systems with different solvent bases. J Adhes Dent 2003;5:183-192.
27. Ruyter IE, Svensen SA. Remaining methacrylate groups in composite restorative materials. Acta Odontol Scand 1977;36:75-82.
28. Sanares AM, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. Dent Mater 2001;17:542-56.
29. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho RM, Pashley DH. Relationship between surface area for adhesion and tensile bond strength-evaluation of a micro-tensile bond test. Dent Mater 1994;10: 236-240.
30. Sakaguchi RL, Peters MC, Nelson SR Douglas WH, Poort HW. Effects of polymerization contraction in composite restorations. J Dent 1992;20: 178-182.
31. Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin-dentin bonding as an array. J Dent Res 1999; 78:699-705.
32. Tay FR, King NM, Suh BI, Pashley DH. Effect of delayed activation on light-cured resin composites on bonding of all-in-one adhesives. J Adhes Dent 2001;3:207-225.
33. Watanabe LG, Marshall GW, Marshall SJ. Variables influence on shear bond strength testing to dentin. In: Tagami J, Toledano M, Prati C (eds). Advanced Adhesive Dentistry – 3rd International Kuraray Symposium, 1999:75-90.
34. Yoshiyama M, Carvalho RM, Sano H, Horner J, Brewer PD, Pashley DH. Regional bond strengths of resin to human root dentin. J Dent 1996;24: 435-442.
35. Yoshiyama M, Carvalho RM, Sano H, Horner J, Brewer PD, Pashley DH. Interfacial morphology and strength of bonds made to superficial versus deep dentin. Am J Dent 1995;8:297-302.
36. Yoshiyama M, Sano H, Ebisu S, Tagami J, Ciucchi B, Carvalho RM, Johnson MH, Pashley DH. Regional strengths of bonding agents to cervical sclerotic dentin. J Dent Res 1996;75:1404-1413.
37. Yoshiyama M, Urayama A, Kimochi T, Matsuo T, Pashley DH. Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. Oper Dent 2000;25:163-169.

Direct clinical relevance: None, since this paper deals with experimental methodology. However, the clinician should know that varying the experimental conditions may have an influence on bond strength results. Therefore, clinicians should ask for the details of the materials and methods when they are confronted with in vitro data by sales personnel.