Dental Materials (2004) **20**, 579–585

dental
materials

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Influence of c-factor and layering technique on microtensile bond strength to dentin

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Received 7 January 2003; received in revised form 22 July 2003; accepted 12 August 2003

KEYWORDS
Dentin bonding; C-factor; Resin composites; Flowable composites; Filled adhesives

Summary *Objective*. Due to polymerisation shrinkage of resin-based composites, a high configuration factor in deep Class I cavities leads to a certain amount of stress when the material is bonded. The aim of this in vitro study was to evaluate the influence of c-factor and different layering approaches on bonding to dentin with three different adhesive systems.

Materials and methods. Dentin bond strengths of Z250 bonded with OptiBond FL, Single Bond, and One Up Bond F were measured on flattened dentin surfaces without cavity walls and on the cavity floor of Class I cavities (10 layering concepts). The resin composite increments were applied horizontally, vertically and obliquely, both with and without a flowable liner. The tests were carried out in a microtensile apparatus at a crosshead speed of 1 mm/min after 24 h of storage at 37 °C in water. Mean bond strengths were analysed using the Wilcoxon test and multiple comparisons according to the Mann–Whitney *U*-test. Specimens having failed prior to the bond strength test were included as 0 MPa.

Results. The groups bonded on flat surfaces exhibited significantly higher bond strengths than specimens cut from filled cavities. Within the cavity groups, OptiBond FL and Single Bond exhibited no significant differences, however, being above One Up Bond F. Within the groups of each adhesive, major differences between the layering concepts were detectable. Bulk technique led to low dentin adhesion at the cavity floor, above all for Single Bond and One Up Bond F. Horizontal layers resulted in significantly higher bond strengths than did vertical or oblique. Lining with a flowable composite did not promote bond strength for OptiBond FL. For the other adhesives, a lining improved adhesion when vertical or oblique layers were applied, for horizontal increments no effect was evident.

Conclusions. The c-factor is an influencing factor for dentin adhesion. However, using an appropriate layering technique, high bond strengths to deep cavity floors can be achieved.

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doi:10.1016/j.dental.2003.08.001

Introduction

In contrast to the situation in the 1980's, tooth-colored materials such as resin-based composites are today the materials of choice for most of the dental patients instead of, e.g. amalgam.[1-3] Due to polymerisation shrinkage of these materials, successful adhesion to enamel and dentin is an indispensable prerequisite for clinical success,[4-6] otherwise gap formation would endanger the clinical success. On the other hand, polymerisation shrinkage stress is only present when shrinking materials are bonded.

Enamel bonding is meanwhile accepted as clinically strong and durable, because acidic etchants, such as 30-40% phosphoric acid, create enamel microporosities allowing the penetration of monomers consecutively generating micromechanical retention.[2,7] In contrast, dentin is an unpredictable substrate for adhesion due to facts like tubular structure, high organic content, and intrinsic wetness.[8-10]

To solve the problem, different approaches are reported in the literature of the field. The two main approaches today are still adhesives with total etching and adhesives with self-etching primers, both delivered as all-in-one systems or with different bottles. Etching with different acids and subsequent penetration of reactive primer molecules into the more or less decalcified dentin surface is recommended.[11-14] While one-bottle adhesives combine primer and bonding resin in one solution, conventional two-bottle systems have a separate primer acting as penetration promotor for the later applied bonding agent.[2,15]

Polar solvents, like acetone or ethanol, are often used as both water chasers and carriers of the monomers to form a tight contact with the exposed collagen network.[16,17] Hybridising adhesive systems were reported to generate high bond strengths to dentin in several studies.[18-23]

Filled adhesives like OptiBond FL and OptiBond Solo Plus (Kerr, Orange, CA, USA), and Permaquik (Ultradent Products Inc., Salt Lake City, UT, USA) form thicker layers and were reported to enhance marginal and internal adaptation of resin composite restorations.[10,15,16,24,25] This may be due to the fact that an oxygen inhibition of the bonding agent is less problematic in thicker adhesive layers. In contrast, unfilled adhesives form thinner layers, which may not be polymerised due to oxygen inhibition.[15]

The majority of studies dealing with dentin bond strengths were carried out on flat bonding surfaces.[13,15,18,21,26,27] Only few data regarding different c-factors are available that indicate the negative potential of cavity geometries on dentin bond strengths.[19,20,24] The c-factor is defined as the quotient between bonded and unbonded resin composite surface resulting in higher values for deeper cavities and vice versa.[5]

Armstrong et al. previously investigated microtensile bond strength and failure characteristics in a high/low c-factor design.[24] A similar approach was published by Nikaido et al., having also reported that the presence of a high c-factor corroborates dentin bond strength to the cavity floor.[19,20] However, several questions are not answered, above all the influence of different layering concepts as meticulously analysed by Lutz et al.[29]

Besides the influence of different c-factors, the effect of different adhesives on dentin bonding performance is still not completely understood. Therefore the present study was additionally carried out with different adhesive approaches, i.e. two-step etch and rinse, three-step etch and rinse, and self-etch 'all in one'.

Altogether, the goal of the present in vitro investigation was to evaluate the effect of different c-factors and incremental techniques and three adhesives by using a microtensile bond strength test (μ-TBS).

Materials and methods

Specimen preparation and microtensile testing

A total of ninety-nine caries-free human third molars were used in this investigation. The teeth were stored in 0.1% thymol solution at ambient temperature for less than four weeks after extraction and were consecutively debrided and examined to ensure that they were free of defects.

For low-c-factor groups, the occlusal enamel was removed by slow-speed diamond-saw sectioning (Isomet, Buehler, Lake Bluff, IL, USA) under profuse water cooling (Fig. 1). A standardised smear layer was created on the surface by wet-sanding with 600-grit sandpaper for 60 s.[28]

For high-c-factor groups, parallel $4 \times 4 \times 4\ \mathrm{mm}^3$ occlusal cavities were cut using coarse diamond burs under profuse water cooling (80 μm diamond bur, Two-Striper Prep-Set, Premier, St Paul, USA), and finished with a 25 μm finishing diamond. Inner angles of the cavities were prepared rectangularly. To guarantee a rectangular relation between the bonded interface and the direction of the later cut μ-TBS beam, the cusps were flattened 2 mm and then the cavity floor was prepared parallel to the flattened cusps (Fig. 1).

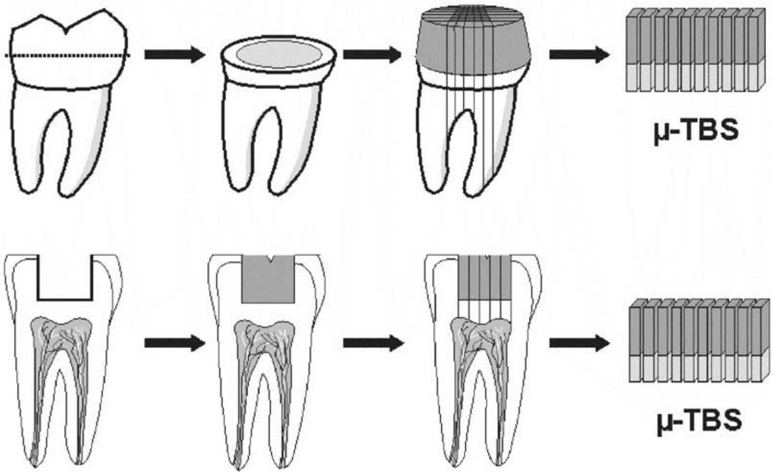

Figure 1 Schematic overview of specimen preparation with low c-factor (upper half) and high c-factor (lower half).

Thirty-three teeth were randomly assigned to one of three dentin adhesives. The teeth were furthermore subdivided into eleven groups with c-factors of ~0.2 and ~4.0. The surfaces were treated with the adhesive systems OptiBond FL (Kerr), Single Bond (3M ESPE, St. Paul, MN, USA) and One Up Bond F (Tokoyama, Tokyo, Japan) according to the manufacturers' instructions (Table 1).

The crowns of the flattened teeth were then reconstructed with four 1 mm-layers of a hybrid resin composite (Z250) each layer being light-cured for 40 s with a Translux CL curing unit (Heraeus-Kulzer, Dormagen, Germany).

The Class I cavities were filled in 10 different ways as shown in Fig. 2. In the groups with a lining, a 0.5 mm thin layer of flowable resin composite (Filtek Flow) was brushed onto the cavity walls and floor and light-cured for 40 s.

The intensity of the light was checked periodically with a radiometer (Demetron/Kerr, Danbury, CT, USA) to ensure that an intensity of 400 mW/cm^2 was always exceeded.

The peripheral areas of the reconstructed/filled teeth were removed resulting in a 4 × 4 mm^2 square central area. The remaining specimen was sectioned into four slices, which were sectioned again to receive 20 resin-dentin beams.[15,27] The saw was adjusted to steps of 1 mm, due to the thickness of the blade (300 μm) resulting in sticks with a cross-sectional area of 700 × 700 μm^2 (0.5 mm^2). From the resulting 60 central sticks of each group, 20 were selected ($n = 20$). These 20 sticks had to have a remaining dentin thickness to the pulp of 2.0 ± 0.5 mm.

If more than 20 beams were collected with the correct remaining dentin thickness, 20 sticks were

Table 1 Investigated adhesive systems, chemical compositions, and manufacturers.

Adhesive system	Code	Etchant	Primer	Bonding resin	Manufacturer
OptiBond FL	OP	Etchant 37.5% phosphoric acid	Primer HEMA, GPDM, MMEP, ethanol, water, initiators	Adhesive bisGMA, HEMA, GPDM, barium-aluminum borosilicate glass, disodium hexa-fluoro-silicate, fumed silica	Kerr Orange CA, USA
Single Bond	SB	Etchant 36% phosphoric acid	Primer + adhesive bisGMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiator, 3-8% water, ethanol		3M ESPE, St Paul MN, USA
One Up Bond F	OF	Mixed all-in-one adhesive Mac-10, phosphate monomer, MMA, bis-MPEPP, HEMA, FASG			Tokoyama Tokyo Japan

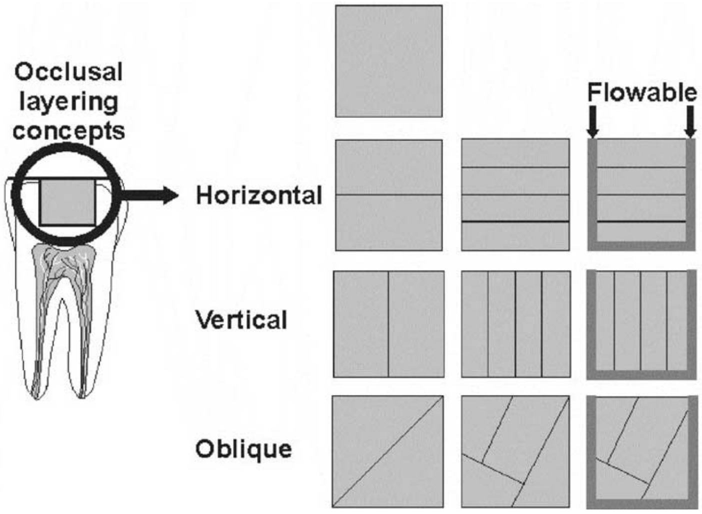

Figure 2 Layering concepts for the high c-factor groups.

randomly selected. For the case that one or more of the selected sticks failed due to the sectioning process, the percentage of prematurely failed specimens in relation to the total number of selected specimens was recorded. The same (or approximated) percentage of the 20 final specimens received 0 MPa as final μ-TBS result.

The μ-TBS beams were stored in distilled water for 24 h at 37 °C.

The sticks were mounted in a Zwick jaw device (Zwick, Ulm, Germany) with a special cyanoacrylate glue (Zapit, Dental Ventures of America, Corona, CA, USA) and tested using an universal testing machine model 4411 (Zwick, Ulm, Germany) with a 50 N load cell traveling at a crosshead speed of 1 mm/min. μ-TBS was determined by computing the quotient of maximum load (N) and adhesion area.

Statistical analysis

The statistical analysis was performed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). The values were non-normally distributed (Kolmogorov-Smirnov test), therefore non-parametric tests were used to compute differences between dependent groups (Wilcoxon matched-pairs signed-ranks test) and among independent groups (Mann-Whitney *U*-test, correction method according to Bonferroni-Holm) for pairwise comparisons at the 0.05 level of significance.

Results

An overview of the results is shown in Tables 2 and 3.

Bond strengths on flat surfaces were 47 MPa for OP, 46 MPa for SB, and 23 MPa for OF (Table 2) being statistically lower than OP and SB ($p < 0.05$; Mann-Whitney *U*-test).

The bond strengths in Class I cavities showed groups being lowered by pre-test failures having been included as 0 MPa. For the bulk fill technique, OP and SB showed significantly higher values than OF ($p < 0.05$; Mann-Whitney *U*-test). Applying horizontal layers exhibited bond strength as a function of the number of increments (Table 3). OP and SB had a similar performance with ~15 MPa for two horizontal layers and ~30 MPa for four increments, being significantly higher ($p < 0.05$; Wilcoxon test). The use of a flowable composite as lining was not beneficial for bond strengths in the groups with horizontal layering.

The groups with vertically applied layers showed worse results compared to the other layering techniques (Table 3). In these groups, bond strengths were improved for SB and OF when a flowable was utilised ($p < 0.05$; Mann-Whitney *U*-test). For SB and OF, the number of PTFs was quite high with vertical layering, e.g. 60% when OF was used with two vertical increments.

Table 2 Results of dentin microtensile bond strength on flat surfaces. Same superscript letter indicates no statistical difference ($p < 0.05$; Mann-Whitney *U*-test).

	μ-TBS to flattened dentin (MPa) (SD)
OP	47.2 (14.8)^A
SB	45.8 (17.4)^A
OF	23.2 (15.1)^B

Table 3 Results of dentin microtensile bond strength to the cavity floor of deep Class I cavities.

	μ-TBS to floors of Class I cavities (MPa) (SD)									
	Bulk	Horizontal layers			Vertical layers			Oblique layers		
		2	4	4^a	2	4	4^a	2	4	4^a
OP	11.8^{BC}	16.0^{B}	31.6^{A}	30.2^{A}	15.7^{B}	18.1^{B}	19.4^{B}	17.6^{B}	16.9^{B}	19.8^{B}
	(8.8)	(5.6)	(8.2)	(8.4)	(6.7)	(6.9)	(8.1)	(12.0)	(10.6)	(7.7)
PTF (%)	15	0	0	0	0	0	0	25	10	0
SB	8.7^{C}	12.7^{BC}	29.9^{A}	30.5^{A}	8.6^{C}	9.8^{C}	18.8^{B}	10.7^{BC}	15.6^{B}	19.5^{B}
	(10.9)	(13.5)	(8.1)	(10.2)	(12.3)	(14.0)	(8.0)	(12.7)	(15.9)	(10.0)
PTF (%)	55	45	0	0	55	60	0	55	45	5
OF	5.0^{D}	14.8^{BC}	16.9^{B}	17.6^{B}	7.0^{C}	12.4^{BC}	18.9^{B}	6.5^{CD}	12.4^{BC}	15.5^{B}
	(11.3)	(14.9)	(15.4)	(14.2)	(10.0)	(15.0)	(11.2)	(10.8)	(14.6)	(13.2)
PTF (%)	80	45	45	25	60	50	15	70	50	20

[a] With lining. PTF: Pre-test failures (%) for each group. Same superscript letter indicates no statistical difference ($p < 0.05$; within materials: Wilcoxon test; between materials: Mann-Whitney *U*-test).

For the groups layered obliquely, a tendency was found in favor of the flowable lining method, however, not being statistically significant ($p > 0.05$).

Discussion

The objective of this in vitro study was to clarify the influence of three adhesives and layering techniques on bond strength to dentin when different configuration factors were present. The c-factors given by the cut flattened surfaces and the cavities do not automatically determine the really existing individual c-factors of each applied increment of resin composite. On the other hand, the crucial first layer of resin composite exhibited similar c-factors in the cavity groups which did not result in similar bond strengths.

The method utilised for bond strength testing was the microtensile bond test reported to be well-suited for the evaluation of bond strengths to enamel and dentin.[22,23,26] This methodology gives the opportunity to investigate interfacial bond strengths on small areas below 1 mm^2. Macro shear and tensile bonding procedures work with larger bonding areas with diameters of up to 5 mm.[26,30,31] When dentin is used as a bonding substrate to evaluate adhesive systems reaching critical bond strengths over ~15 MPa, shear and tensile procedures tend to produce non-uniform stresses during the debonding process resulting in cohesive fractures in dentin.[30,31] However, cohesive fractures in dentin do not represent the clinically relevant failure mechanism in real cavities. This particular problem can be prevented with microtensile testing because the predominant failure is also adhesive during the present investigation. This confirms the findings of other studies dealing with microtensile testing.[21,28] Shono et al. reported in recent investigations using the μ-TBS methodology, that bonding to flattened dentin surfaces exhibits differences at variable distances from the pulp, potentially resulting in regional dentin bond strength differences.[22] Therefore, only the very central areas of the specimens were used to obtain a reliable randomisation of test specimens like in previous studies.[15,27]

According to the suggestion of Pashley et al., statistics have been carried out with $n = 20$ as number of specimens under investigation per experimental group.[21]

Several studies excluded specimens that failed during the sectioning process. This may be wrong, because there may have been a certain amount of force to produce the failure during sectioning, so one could estimate this individual force as more than 0 MPa. Another approach is to count the individual number of so-called pre-test failures (PTF).[32] This definitively is an honest way to qualitatively describe some deficiencies. However, when e.g. in the case of a weaker adhesive 50% of the beams of one tooth cannot be tested due to early failure during the machining process, the other 50% of specimens may not really represent a realistic bond strength image of the adhesive potential of the particular adhesive tested.

Early failures during specimen sectioning have been frequently observed especially in deep cavities during the present study. Therefore, the authors decided to analyse differently in comparison to previous studies resulting in lower bond strength values. Furthermore this illustrates that some problems in deep cavities may arise from a clinical point of view. So the inclusion of 0 MPa-specimens had a strong influence on the results of the present study: Placing the composite in bulk resulted in 5 MPa according to the protocol of this study, however, conventional statistical analyses would have revealed 25 MPa. A more detailed view is given in Table 3 displaying the influence of different percentages of prematurely failed specimens in different groups under investigation.

For an additional fatigue simulation, chewing simulation of the involved specimens would have been possible[19,20] to further stress the bonded joint between resin and dentin. This could be the goal of further investigations for more optimisation of the layering concept. Also long-term storage space could affect the results.[23,32] However, the present study showed, e.g. a 45% PTF rate for OF with two horizontal layers which is already poor and alarming without additional fatigue or long-term storage approaches to obtain evidence about resin-dentin bonding of contemporary adhesive systems.

An important point for dentin bond strengths is the individually formed thickness of the bonding agent.[15,33,34] The thicker adhesive layers in the OptiBond FL control groups may have contributed to slightly higher dentin bond strengths. However, there was no statistical difference to Single Bond. This observation is reflected by the work of Armstrong et al., not having found differences in cavities for OptiBond FL and an unfilled experimental version of OptiBond FL also tending to reject the elastic cavity wall theory.[24]

On the other hand, Choi et al. reported that thicker layers of an adhesive providing a low modulus of elasticity are capable of reducing the interfacially acting polymerisation stress of resin composites.[35] When Class I cavities are involved, not only the c-factor but also the characteristics of deep dentin as adhesion substrate may contribute to lower bond strengths.[36,37]

The use of lining materials providing a lower modulus of elasticity may result in inhomogeneous stress distribution near the bonded interface.[38]

It is a well-known fact from the literature that a high c-factor is a risk factor for bonding because polymerisation stresses may be enlarged. Feilzer et al. showed that under simulated clinical circumstances the resulting stress may be too high to be counteracted by dentin bonding.[39]

On the other hand, bulk application may not allow sufficient light polymerisation of the solely light-curing materials. Therefore layering concepts have been described as mandatory when working with resin-based composites. The different results displayed in Table 3 demonstrate that there are significant differences in bond strengths to the cavity floor when different incremental techniques are applied in deep Class I cavities.

Comparing the different layering concepts, it was shown as crucial, *where* the first increment is bonded. Only when the first increment has been bonded to the cavity floor, acceptable bond strengths and percentages of 0 MPa-specimens were observed (Table 3). The vertically and obliquely layered fillings frequently exhibited almost no adhesion of the restorative to the cavity floor. This may have contributed to the fact that a tight adaptation with a plugger can be achieved more easily in a horizontal than a strictly vertical way, potentially leading to voids within the stressed interface.

With the different adhesives used in the present investigation, a clear advantage for OptiBond FL and Single Bond is evident from the bond strength results. In the OP groups even with vertical and oblique layering acceptable bond strengths combined with few 0 MPa-specimens resulted in the cavity tests. Single Bond and One Up Bond F performed worse than did OF when few layers were applied into the Class I cavities. Furthermore, it would be interesting to learn how the applied layering concepts influence dentin adhesion to the vertical cavity walls. This is the aim of further studies.

Compared to the results of Nikaido et al.,[20] SB revealed higher values for μ-TBS. Nikaido et al. found overwet zones responsible for the poor performance of SB concluding this particular adhesive to be technique-sensitive. According to findings from a previous study,[27] the intrinsic moisture in SB seems to be sufficient to avoid too much water left in the cavity. On one hand this confirms the terminus technique sensitivity, on the other hand the results are not comparable with the present investigation, because 0 MPa-specimens were not excluded.

Altogether, SB and OP revealed better overall dentin bonding performance than the simplified bonding agent OF. A solely self-etching system may provide less technique sensitivity due to the absence of total etch/wet bonding requirements, however, the more old-fashioned adhesive systems OP and SB produced more promising results overall.

Conclusions

- The presence of a high c-factor is a risk for debonding within the resin-dentin interface.
- For deep Class I cavities, horizontal layering is the most promising way to get a good bond to the cavity floor.
- Using four horizontal increments, OptiBond FL and Single Bond achieved higher bond strengths than One Up Bond F.

- For Single Bond and One Up Bond F, lining with a flowable composite improved bond strengths when vertical layers are applied.

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