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Method to compare μ-tensile bond strength of a self-etching adhesive and μ-cohesive strength of adjacent dentin

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Summary Laboratory results from tensile or micro-tensile testing of adhesive/restorative systems need a gold standard to make their interpretation possible. This can be done by comparing the μ-tensile bond strength (μTBS) of the tested adhesive(s) with the μ-tensile cohesive strength (μTCS) of the adjacent dentin, which is meant to be replaced by the restoration.
Objectives. To test immediate μTBS of an adhesive/restorative system versus μTCS of the dentin adjacent to the bonded interface, in perfused specimens.
Methods. Enamel and roots of five-third sound molars were removed and teeth were perfused (30 cm distilled H_2O) until after the bonding procedure was completed. Parallel grooves (1.5-mm wide × 1.5-mm deep, separated by 1.5 mm), were drilled in the exposed dentin, under water refrigeration. Teeth were restored (AdheSE/Filtek Z250). Specimens were sawn from grooves to test μTBS ($n=30$) and from between grooves to test μTCS ($n=32$) of dentin, adjacent to adhesive interfaces, both areas to be tested at the same relative depth. Areas to be studied were rounded (mean bonded surface area for both groups, BA = 0.68 mm^2), and μTBS and μTCS were found. As not all BAs were identical, residuals of μTBS and μTCS values to the regression line relating BA and tensile test results (representing the null hypothesis, i.e. μTBS = μTCS) were compared using a non-parametric test.
Results. Difference in the means of residuals was not statistically significant (two tails $p=0.067$).
Significance. mean μTBS of the tested adhesive was not different to mean μTCS of adjacent dentine. The null hypothesis was not rejected.

Introduction

The determination of bond strength of dental adhesives to hard dental tissues is a matter of importance, in accordance with the great changes that adhesive techniques have produced in

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dentistry [1]. Usually, this bond strength is determined measuring the tensile or shear load at failure [2] between the adhesive and the substrate, divided by the cross-sectional area (BA) of the bonded interface to be tested [3].

In vitro tensile bond strength (TBS) results are influenced by a high number of variables [2,4–9], including: time elapsed from extraction until the measuring procedure starts, tooth type (carious/sound, young/adult, erupted/unerupted) and pretreatment (storing medium and time, temperature), their handling (perfusion yes/no, wetness) during and after (mechanical/thermal cycling, embedding) testing, tooth origin (human/animal), depth and location of interface, mechanical characteristics of the restorative material used, type of test (tensile, shear-pure or push-out-, micro-tensile, micro-shear), its stress rate, and specimen design, largely BA range and shape.

This variability originates great scattering in results obtained in different laboratories [4,8, 10,11], making necessary a standardization of bonding tests. However, total standardization of a bonding test seems problematical because not all laboratories would have the same facilities, use the same type of specimens, and follow the same protocols.

A reasonable alternative is to define a *gold standard*, a consensus value for comparison. This gold standard should represent an ideal or, at least, desirable TBS level that could be universally acceptable. If such a value could be established, it could become possible to level TBS values from different laboratories against it.

Many of the problems in comparing results from different laboratories come from testing adhesive restorative systems used on dentin. In this situation, an ideal material would theoretically be one that produces a bonded interface having comparable properties to the ones of the dentin it is replacing. In other words, TBS of the adhesive interface should, ideally, be as close as possible to the cohesive strength (CS) of the dentin it is substituting.

Actually, value of this gold standard is not universal because the CS of dentin also depends on the method used to measure it. Nevertheless, CS can be verified each time an adhesive is tested, under the same circumstances, in the same specimens and at the same or comparable location. In this way, the variable factors that depend on the research protocol and affect adhesion and cohesion testing, depending on each laboratory procedures, would influence both values (TBS and CS of dentin) simultaneously and the CS of dentin could be used as a gold standard.

Adhesive complex (one or more uneven layers of intact, demineralized and infiltrated dentin or enamel, of adhesive, and of restorative material, that are formed when adhesive procedures are undertaken) is not uniform [9]. It can have disruptions, gaps or air bubbles, and is formed over an irregular surface, the dentin or the enamel. This produces an irregular stress distribution [12]. When relatively large bonded areas (BAs) are used to test TBS of an adhesive interface, the probability of one or more disruptions being included in them is high, this disruptions then possibly acting as fracture initiators, causing low apparent TBS values [9,13]. If this is the case, results are lower than if smaller BAs would have been used. This would not be a major inconvenience if all laboratories would use the same BAs throughout their experiments, but this is far from happening.

To diminish this inconvenience, the micro-tensile bond strength (μTBS) test was developed [14]. When it was first used, BA ranged from 0.25 to 9 mm^2 [15]. Since then, several studies have been published [16–24] that used BA of or around 1 mm^2, resulting in an increase in published TBS results.

Furthermore, there are some studies [15,25–27] that show the relationship between BA and TBS to be somehow inversely related (Fig. 1). Mathematical description of their regression curves is shown in Table 1. The first of them [15] shows the relationship to be logarithmic, the second [25] to be linear, the third [26] to be inverse and the fourth [27] to be exponential. All of them show that smaller BAs bring out higher μTBS, whatever the relation would be. And, with the exception of one of them [25], all show that this relation is not directly proportional: μTBS value increases faster than BA decreases, when BA is close or below 1-mm^2. This makes the discrimination of BA critical when small testing areas (circa 1 mm^2) are used.

In comparative studies, where BA will not be kept exactly constant for all specimens, there is then an intrinsic problem in comparing data within the study, which will cause distortion in the final results. Although BA means of all groups may be very similar or equal, this does not necessarily illustrate that it is identical for all samples. It may well happen that some of the specimens are tested using larger areas than others. And, as we have seen, minor differences in BA will, when areas around 1 mm^2 are used, produce very high divergences in μTBS results. In this situation, one or more groups may have an advantage when compared with others, because their apparent μTBS results will be higher.

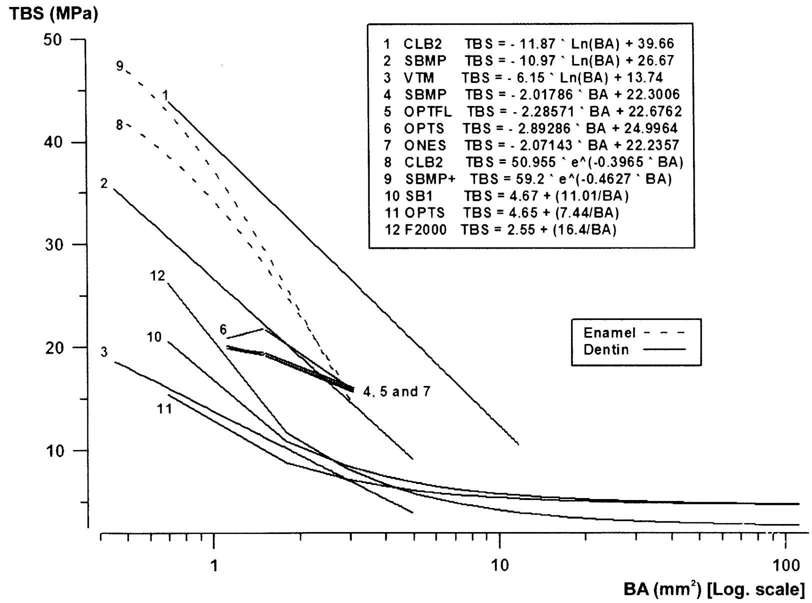

Figure 1 Previously described relationships between BA and TBS results. Note Logarithmic scale in BA axis.

One proposed solution to this [16,28] is, using the least-squares means test, to adjust for the bonded area, before comparing bond strengths.

Regardless of what the mathematical expression is that will better describe the relationship between BA and μTBS in each experiment, this is usually calculated by the least-squares method. In this method, the total sum of the residuals (differences between the actual μTBS value and the value predicted by the formula) is minimal or, if perfect adjustment is possible zero. These residuals are the distances from each individual sample results to the regression curve. Because of the calculation method, some of them are positive (i.e. μTBS result for that case is higher than predicted) and some negative.

In this scenario, it can be assumed that the regression curve represents the behavior of all the materials that are being compared, as a group, whatever the individual BA is. Statistically, this line is the representation of the null hypothesis: if all specimens (of all materials) had the same μTBS, all of then will be exactly located along the line.

In the simplest situation, when two materials' μTBS (or a material's μTBS and dentin's μTCS, as in this report) are compared, if one of them has

Table 1 Previously published relationship among BA and TBS.

Authors	Material	BA range		Proposed mathematical function	Substrate	Perfusion
		Max	Min			
Sano et al. [15]	CLB2[1]	11.65	0.7	$TBS = -11.87 \times Ln(BA) + 39.66$	Dentin	No
	SBMP[2]	4.95	0.45	$TBS = -10.97 \times Ln(BA) + 26.67$		
	VTM[3]	4.95	0.45	$TBS = -6.15 \times Ln(BA) + 13.74$		
Prukkanon et al. [25]	SBMP[4]	3.1	1.1	$TBS = -2.01786 \times BA + 22.3006$		
	OPTFL[5]			$TBS = -2.28571 \times BA + 22.6762$		
	OPTS[6]			$TBS = -2.89286 \times BA + 24.9964$		
	ONES[7]			$TBS = -2.07143 \times BA + 22.2357$		
Shono et al. [27]	CLB2[8]	3	0.5	$TBS = 50.955 \times exp(-0.3965 \times BA)$	Enamel	
	SBMP+[9]			$TBS = 59.2 \times exp(-0.4627 \times BA)$		
Macorra et al. [26]	SB1[10]	110.86	0.69	$TBS = 4.67 + (11.01/BA)$	Dentin	Yes
	OPTS[11]			$TBS = 4.65 + (7.44/BA)$		
	F2000[12]			$TBS = 2.55 + (16.4/BA)$		
	All[13]			$TBS = 4.17 + (10.35/BA)$		

BA, bonded area (mm^2); TBS; tensile bond strength (MPa); CLB2; clearfil liner bond 2; SBMP, ScotchBond multi-purpose; VTM, vitremer; OPTFL, optibond FL; OPTS, optibond solo; SBMP+, scotch bond multi-purpose plus; SB1, ScotchBond 1; Ln, natural logarithm; e, 27,183. Numbers in superscripts in the material's column identify each curve in Fig. 1. Numbers in the author's column identify references.

a higher mean than the other, its results will, on average, be above the regression line, i.e. the mean of its residuals would be positive. The other's material residuals mean will be under the line, the mean of its residuals being negative.

Statistically, this means that the material with a positive residuals mean has higher results than it would have if the null hypothesis was true, and the second one has lower ones. In this way, comparing the means of the residuals for both materials with a simple test will easily and accurately test this hypothesis.

Objectives of this work are, first: to introduce and to test the micro-cohesive strength (μTCS) of dentin as a *gold standard* to level μTBS of a current self-etching adhesive and, second: to test the hypothesis that the self-etching adhesive has similar μTBS than μTCS of adjacent dentin. (Null hypothesis, μTBS = μTCS).

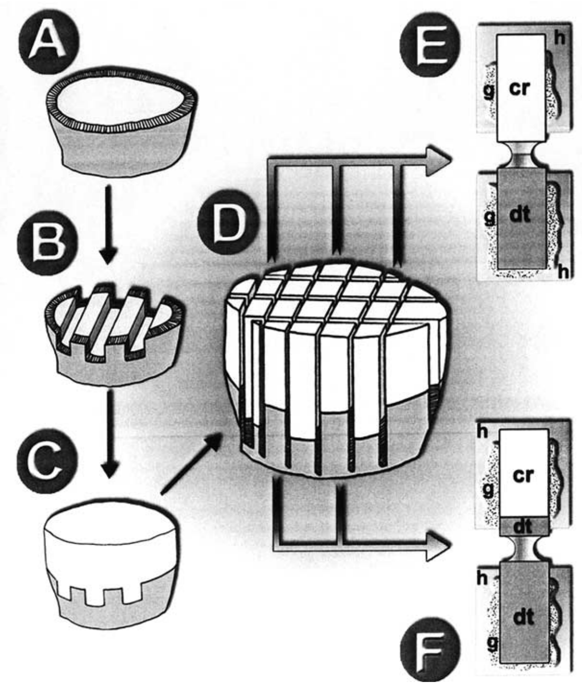

Figure 2 (A) Coronal and apical sectioning of specimen. (B) Grooves drilled in the occlusal aspect. (C) Composite buildup. (D) Two-planes specimen sectioning, parallel and perpendicular to grooves. (E) Glued rounded sticks from grooves to induce fracture at the interface level, to measure μTBS. (F) Glued rounded sticks from between grooves to induce fracture at the same relative dept than in E, to measure μTCS. cr, composite resin; dt, dentin; g, glue; h, holder.

Material and methods

Five-third lower molar teeth were sectioned (Exact 300 CP, Exact Apparatebau GmbH & Co, Nordestedt, Germany) at occlusal and furcation level (Fig. 2A). The contents of the pulpal chamber were carefully removed with cotton and pliers. Apical aspects were bonded to methylmethacrylate cubes that allowed passing of two catheters into the closed pulpal chamber. These catheters were connected to a perfusion system, thus forming an artificial pulpal chamber that was completely filled, to fully rehydrate specimens. A constant pressure in the system (30 cm distilled H_2O) was maintained during the whole of preparation and bonding procedure.

An appropriate number of parallel grooves (1.5 mm deep, 1.5 mm wide, with 1.5 mm of separation) was drilled, with constant abundant water-cooling, on the occlusal aspect (Fig. 2B).

The adhesive to be tested (AdheSE, Ivoclar Vivadent, Schaan, Liechtenstein, lot E35150) was applied and a resin composite (Filtek Z250, 3M ESPE, Seefeld, Germany, lot 2TF/A3) buildup was made (Fig. 2C) in two increments, one to fill grooves and a subsequent one to complete the buildup.

Specimens were vertically sectioned in two planes: parallel to the grooves and at 90° to them (Fig. 2D), obtaining a variable number of compound rectangular sticks (dimensions $\approx 1.2 \times 1.2$ mm) from the grooves (to measure μTBS) and from between the grooves (to measure μTCS). All sticks were rounded to induce fracture at a similar relative depth, i.e. where the interface was placed in the group obtained from the bottom of the grooves. The diameter at the site of induced fracture was measured (Mitutoyo Digital Caliper), in order to find the BA (mm^2).

Specimens were glued (Rocket, Dental Ventures of America, Corona, CA, USA) (Fig. 2, E—for μTBS- or F—for μTCS-) to a custom-made specimen holder. Vertical load at fracture (N) was measured in a tensilometer (Hounsfield 500N, Croydon, England) at a crosshead speed of 1 mm/min, and μTBS and μTCS were calculated (MPa).

μTBS and μTCS results were related to their respective BAs (SPSS 11, Chicago, IL, USA) through a non-linear regression, with the least-squared method (Fig. 3). Residuals (MPa) of each actual case were stored in the variable 'distance'. The regression line was assumed to represent the null hypothesis, i.e. if all cases of both measurements had been identical, they would all had lain along this line.

As the dentin's source may have had an influence, this study separately tested if μTBS and μTCS results were homogeneous for different teeth. The Shapiro-Wilk test for normality was used to test if

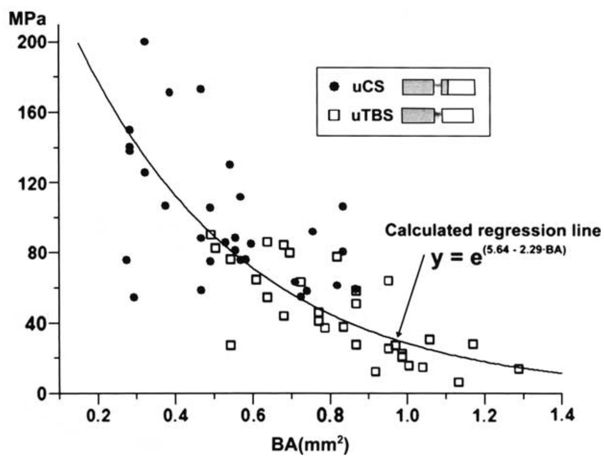

Figure 3 Experimental relationship between BA magnitude (in mm^2) and μTBS (empty squares, in MPa) and μTCS (filled circles, in MPa), and the corresponding calculated regression line.

μTBS and μTCS distances followed a normal distribution, and the ANOVA test was used to compare μTBS and μTCS of different sources, using tooth origin as the independent variable.

To test the null hypothesis, a Shapiro–Wilk normality test was applied to find out if the distribution of 'distance' values was normal in both μTBS and μTCS groups, and a two-tailed non-parametric Mann–Whitney *U* comparison test was applied to test if the difference between the mean of distances (μTBS and μTCS) was statistically significant.

Results

Results for BA, μTBS, μTCS and calculations of distances are shown in Table 2. One mean of distances (μTCS, 9.02 MPa) was positive and the other one negative (μTBS, −0.76 MPa), showing that groups are different. For each of them, specimens of the first are located, on average, over the common regression line and specimens of the second, below it.

It can be observed that the mean BA is higher in μTBS specimens (0.84 mm^2) than in μTCS specimens (0.53 mm^2). It was not possible (nor intended), in this experiment, to obtain exactly the same BA of all specimens, as this would have required very expensive equipment and more manipulations.

Standard deviation of the variable distance is relatively high, because the values are of the vertical distance in each case to the regression line, ignoring the distance from the line to zero at each point.

To test if tooth origin of specimens had an influence on results, we compared μTCS and μTBS means within teeth. ANOVA showed that differences were statistically not significant ($p = 0.476$ and 0.339, respectively). This supports the assumption that tooth origin had a negligible influence in this study.

Relating tensile test results (both μTCS and μTBS) with their individual BAs with non-linear regression, results were: results = exp(5.64 − 2.29 × BA), with an adjusted $r^2 = 0.63$ ($p < 0.00001$). Regression explains 79% of the variation in tests results. From the graph the importance of considering BA can be seen, since μTCS was calculated on specimens with smaller areas than the ones used to find μTBS.

The Shapiro–Wilk test for normality of distribution of distances showed that μTCS did show a normal distribution ($p = 0.068$), but μTBS did not ($p = 0.014$). The Mann–Whitney *U* comparison test results showed that the μTCS mean distances and the μTBS mean distances' difference (9.02 − 0.76 = 9.78) were not statistically significant (exact bilateral $p = 0.067$).

Discussion

μTBS and μTCS were obtained, respectively, on specimens obtained from grooves and from between grooves prepared in dentin (E and F in Fig. 3, respectively). When the built-up composite resin was applied, grooves were filled up in the first increment. In subsequent increments the rest of the buildup was completed. This might have an influence on results, because the configuration factor for the bottom of the grooves, which supplies

Table 2 Results.

	Dentin			Adhesive		
	BA	CS	Distance	BA	μTBS	Distance
n	30			32		
m	0.53	99.37	9.02	0.84	45.14	−0.76
SD	0.19	38.85	36.68	0.20	25.56	17.39

n, number of cases; *m*, mean; SD, standard deviation; BA, bonded area (mm^2); CS, cohesive strength of dentin (MPa); μTBS, microtensile bond strength (MPa); distance, distance (in MPa) from the actual case to the regression curve.

specimens for μTBS measurements, is higher than the one when buildup was completed and for the commonly used setup, where a flat dentin surface is used.

Specimens were obtained from perfused (30 cm of distilled H_2O) specimens, and this perfusion was maintained during the drilling of grooves and the bonding procedure. It has been shown that perfusion has a detrimental effect on tensile test results of bonded interfaces [7,29,30]. In this instance, it seems not to have major consequences, because the adhesive/restorative combination tested has an acceptable behavior, in terms of these in vitro findings.

Specimens were rounded with a flame-shaped diamond bur. The rounded design of the BA was shown [25] to concentrate the stress at the narrowest part of the specimen, where the fracture was induced to move across the bonded interface, in μTBS measurements. This stress will be evenly distributed from the circumference to the center of the bonded area.

Special attention was given to prepare the area to be tested at the same relative depth in both groups (bottom of grooves of μTBS specimens). Due to the different composition of the area to be tested (adhesive interface or dentin), both groups of specimens were prepared resulting in distinct BAs, to avoid as much as possible premature or spontaneous fractures. Consequences are that mean BA was higher in the μTBS specimens ($0.84\,mm^2$) than in the μTCS specimens ($0.53\,mm^2$). This difference appeared because when μTBS specimens were prepared, rounding of the interface was not as aggressive (range of BA: 1.29-$0.49\,mm^2$), as these specimens tend to split 'spontaneously' and fracture easily, probably because of the difference in stiffness between both sides of boundary. On the other hand, μTCS specimens could be rounded reaching a higher depth (range of BA: 0.87-$0.27\,mm^2$) because of their higher elasticity and homogeneity.

The best non-linear regression is obtained with the growth curve $\mu TBS = \exp(5.64 - 2.29 \times BA)$. This regression explains 79% of the variation of tests results ($r = 0.79$). This type of relationship is different to others previously described [15,25-27], where the relation had other mathematical formulations. In this study, a small range of BAs are used (0.27-$1.29\,mm^2$), forcing a very narrow window to define the relation. It is probable then, that if a wider BA range was used, mathematical expression of this relation would change.

The data in this study showed that the mean μTCS (SD) is 99.37 (38.85) MPa (using areas ranging from 0.27 to $0.87\,mm^2$), and the mean μTBS (SD) is 45.14 (25.56) MPa with the BA ranging from 0.49 to $1.29\,mm^2$. These data were not directly compared because μTCS results (filled circles in Fig. 3) are superior to a greater extent because they were obtained from specimens where the prepared BA was smaller.

When distances to regression line for both (μTBS and μTCS) groups are compared, their difference ($9.02 - 0.76 = 9.78$ MPa) is not statistically significant (bilateral, two-tails, $p = 0.067$). This means that the tested adhesive produces an adhesive interface that has a micro-tensile bond strength statistically not different to the micro-cohesive strength of adjacent dentin.

However, it is important to clarify the meaning of the statistical significance in this report. Although it could have been thought *before* the experiment that μTCS > μTBS, there were no data supporting this assumption. When this is the situation (to the authors' knowledge, there were no previous data on μTBS and μTCS in the same experiment), assumptions about which is the direction of the difference (about if μTCS > μTBS or μTCS < μTBS) cannot be made, and the test has to be arranged two-tailed, bilateral. If the completely different hypothesis μTCS > μTBS had been tested, something that can be made from this experiment (as it can now be assumed that μTCS > μTBS) significance would have been unilateral ($p = 0.067/2 = 0.034$), a radically different result.

Another possible way of relating results is calculating the predicted value for $BA = 1\,mm^2$ for both interfaces. This calculation results in $\mu TCS_{1\,mm} = 37.5$ MPa, and $\mu TBS_{1\,mm} = 27.7$ MPa. These would have (theoretically) been the results for both parameters if it had been possible to prepare all specimens with exactly $BA = 1\,mm^2$. To put it in other figures, the mechanical resistance of the adhesive/restorative system would have reached 74% of the cohesive strength of adjacent dentin if BA had constantly been $= 1\,mm^2$. The relevance of this level of difference still has to be found.

Table 3 shows CS values of previous publications. In these reports only mean BAs are cited, and it was not possible to know if all areas of all specimens throughout each report were the same. It can be seen that the predicted μTCS results in this study for $1\text{-}mm^2$ areas are lower than published. This difference could be caused by the storage method (specimens in the current study were kept in distilled water for up to a month before testing) or by the area where μTCS was measured. Distance to pulpal chamber—remaining dentin thickness—is relatively low in this experiment, because it had to be at the same level as the bottom of the grooves, where μTBS was measured.

Table 3 CS of dentin values previously published.

Authors	Area used (mm^2)	CS (MPa)
Sano et al. [14]	0.25	93.8
Zhang et al. [31]	0.275	20
Carvalho et al. [32]	0.5	57.6
Bowen and Rodriguez [33]	0.97	52
Lehman [34]	1.37	41
Liu et al. [35]	1.5	83.93

The proposed method for comparing results has some advantages. First, the dimensions of the bonded areas do not have to be exactly the same. This is impossible to achieve without complicated—and expensive—equipment and usually requires many specimen manipulations. Even more, this is sometimes undesirable if, for instance, research is designed to compare a brittle material as an adhesive substrate with a more elastic one. In this case, researchers may want to use bigger areas when testing the more rigid material than would be used with a more elastic one.

Second, this method makes it easier to compare results between different laboratories. Usually, results obtained in different laboratories have a considerable scatter due to different protocols of measurement. In this study, a gold standard was used: μTCS of dentin adjacent to the adhesive interface to be tested. All efforts were made to test dentin contiguous to the bonded interface, at the same relative depth. In this way, μTBS results can be compared to a relevant measurement: micro-cohesive strength of dentin of the same teeth, at similar locations and with the same protocol, which is a good reference for what the tensile resistance of the bonded material should be. This comparison can be carried out directly, as in this study: finding the significance of the eventual differences of the means of distances to the common regression line, or by the percentage of μTBS results into μTCS of dentin. This would show what level reaches, in that specific laboratory, the tested adhesive(s).

Conclusions

The adhesive tested showed micro-tensile bond strength lower than micro-tensile cohesive strength of adjacent dentin, in perfused teeth, but this difference was not statistically significant.

The calculated micro-tensile strength of the adhesive/restorative system tested reached 73.8% of the cohesive strength of adjacent dentin, for bonded areas of 1-mm^2.

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