Published in final edited form as:

Oper Dent. 2009 ; 34(2): . doi:10.2341/08-55.

Effect of 2% Chlorhexidine Digluconate on the Bond Strength to Normal versus Caries-Affected Dentin

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SUMMARY

This study evaluated the effect of 2% chlorhexidine digluconate (CHX) used as a therapeutic primer on the long-term bond strengths of two etch-and-rinse adhesives to normal (ND) and caries-affected (CAD) dentin. Forty extracted human molars with coronal carious lesions, surrounded by normal dentin, were selected for this study. Flat surfaces of two types of dentin (i.e. ND and CAD) were prepared with a water-cooled high speed diamond disc, and then acid-etched, rinsed and air-dried. In control groups, dentin was re-hydrated with distilled water, blot-dried and bonded with a three-step (Scotchbond Multi-Purpose-MP) or a two-step (Single Bond 2-SB) etch-and-rinse adhesive. In experimental groups, dentin was re-hydrated with 2% CHX (60 s), blot-

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dried and bonded with the same adhesives. Resin composite build-ups were made. Specimens were prepared for microtensile bond testing in accordance with the non-trimming technique and then tested either immediately or after 6-month storage in artificial saliva. Data were analyzed by ANOVA/Bonferroni tests ($\alpha = 0.05$). CHX did not affect the immediate bond strength to ND or CAD ($p > 0.05$). CHX treatment significantly lowered the loss of bond strength after 6 months seen in control bonds for ND ($p < 0.05$), but it did not alter the bond strength of CAD ($p > 0.05$). Application of MP on CHX-treated ND or CAD produced bonds that did not change over 6 months of storage.

INTRODUCTION

The restorative treatment of carious teeth has undergone profound changes since the outer, non-contaminated layer of carious dentinal lesion was found to be suitable for remineralization¹. Operative procedures have shifted from removal of large extensions of the affected tooth to the completion of more conservative and minimal invasive cavity designs^{2,3}, in which the affected, non-contaminated dentin can be deliberately retained in the preparation.

Contemporary etch-and-rinse and self-etch adhesives play a significant role in the coupling of resin composites to caries-affected dentin by providing reasonable initial bond strength.^{4,5} However, morphological studies have shown that a perfect hybrid layer is rarely formed in caries-affected dentin.^{6,7} This hypomineralized, porous substrate allows deeper penetration of the etchant, leading to a deeper demineralization of dentin.^{4,8,9} With a deeper zone of demineralization, it is more difficult for resin monomers to completely infiltrate the collagen dentin matrix.^{6,7} Accordingly, the prevalence of exposed, unprotected collagen fibrils within the hybrid layers that are formed in caries-affected dentin may be greater when compared to hybrid layers in noncarious dentin.⁶

The durability of resin-dentin bonds made to normal¹⁰, sclerotic¹¹ or caries-affected dentin¹² (i.e. normal or caries-affected), using contemporary simplified adhesives has been shown to be poor, as these adhesives do not perfectly seal the exposed dentin.^{13,14} Moreover, increasing the concentration of ionic and polar comonomers in simplified adhesives to make them more hydrophilic results in increased water sorption^{15–17}, which ultimately lowers their mechanical properties by polymer plasticization.^{15,16,18} Thus, the use of hydrophilic simplified etch-and-rinse adhesives may never achieve the goal of creating a durable coupling between resin composites and resin-bonded dentin.

During caries progression, dissolution of some of the mineral phase of dentin leads to exposure of some of the organic matrix that can then be degraded by bacterial enzymes¹⁹ and by host-derived collagen-degrading proteases^{20–22}, such as the matrix metalloproteinases (MMP) that are found either in saliva^{21,23} or in dentin organic matrix.^{24–26} MMPs are thought to be responsible for degrading most of the extracellular matrix components, including different types of collagen, in native and denatured forms.^{27,28} It has been suspected that the activity of host-derived MMPs may also be involved in the degradation of unprotected collagen within incompletely resin-infiltrated acid-etched dentin^{29–32}, which could explain the progressive thinning of hybrid layers seen in numerous *in vivo*^{30,32,33} and *in vitro*^{10,31} aging studies.

Persistent collagenolytic activity exhibited by unbonded, partially demineralized human dentin substrates, in the absence of bacteria, was recently associated with a morphological disintegration of dentinal collagen fibrils.²⁹ Nevertheless, it was also shown that the dentinal collagenolytic activity can be strongly reduced by the use of chlorhexidine²⁹ a potent MMP inhibitor. Chlorhexidine also prevents or minimizes the auto-degradation of exposed

collagen fibrils within incompletely-formed hybrid layers^{30,32}, thereby contributing to the long-term stability of hybrid layer and bond strength. However, the research has so far been limited to a single two-step, etch-and-rinse adhesive (i.e. Single Bond, 3M/ESPE).

The aim of the present study was to evaluate the effect of a 2% chlorhexidine digluconate primer (CHX) on the durability of resin-dentin bonds in normal *versus* caries-affected dentin, using three-step or two-step etch-and-rinse adhesives. The tested hypotheses were that: 1) CHX does not cause a detrimental effect on the immediate bond strength to either normal or caries-affected dentin; 2) Preservation of bond strength to both normal and caries-affected dentin over time is not adhesive- or CHX-dependent.

MATERIAL AND METHODS

Tooth Preparation

Forty extracted human molars with coronal caries lesions were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the Ethic Committee for Human Studies, Piracicaba School of Dentistry/UNICAMP, Brazil. Selected teeth were stored for 1 month or less in 0.5% chloramine T solution at 4°C. Occlusal enamel and dentin were removed horizontally (i.e. perpendicular to the long axis of the tooth) through the carious lesion, mesio-distally, using a water-cooled diamond-impregnated disc (Buehler Ltd., Lake Bluff, IL, USA) to expose a flat dentin surface, wherein the carious lesion was surrounded by normal, sound dentin. Exposed dentin surfaces were stained with Caries Detector solution (Kuraray Medical Inc., Tokyo, Japan) for 10 sec and rinsed with water. The carious lesion was then manually excavated until there was no more bright-red-staining dentin. Caries-affected dentin was defined as dentin that was colorless to light pink, firm and opaque. The entire dentin surface was then ground flat with 600-grit SiC paper under running water.

Bonding procedures

All dentin surfaces were etched with 35% phosphoric acid gel (Scotch Etchant, 3M ESPE, St Paul, MN, USA) for 15 s, rinsed for 30 s with tap water and vigorously dried with oil/water-free air. Teeth assigned to the control groups were re-hydrated with 15 µL of distilled water, gently blot-dried and bonded with one of the etch-and-rinse adhesives: the three-step Scotchbond Multi-Purpose (MP - 3M ESPE, St Paul, MN, USA) or the two-step Single Bond 2 (SB - 3M ESPE, St Paul, MN, USA), according to manufacturer's instruction. In the experimental groups, dentin was re-hydrated with 15 µL of 2% CHX (PRODERMA, Piracicaba, SP, Brazil). After a dwell time of 60 s, the dentin surface was gently blot-dried and bonded with one of the two adhesives. Three 1.5-mm thick layers of a resin composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) were incrementally placed over the bonded dentin surfaces and individually light-cured for 20s, using a quartz-tungsten-halogen curing-light unit (XL 3000- 3M ESPE, St Paul, MN, USA) with an output of 700 mW/cm². Teeth were stored in 0.02% NaN₃-containing phosphate-buffered saline (PBS, pH 7.4) at 37°C for a week.

Microtensile bond testing

Teeth were longitudinally sectioned across the bonded interface using a water-cooled diamond saw in order to produce a series of 0.9 mm thick slabs. Each slab was then cut into multiple 0.9 mm × 0.9 mm × 8 mm beams. Twelve to fourteen beams were obtained from each bonded tooth. Half of those specimens were immediately tested, while the remaining specimens were stored at 37°C for six months in NaN₃-artificial saliva (pH 7.1) (Table 1) to inhibit microbial growth. The storage medium was replaced weekly. Each beam specimen was individually fixed to a custom-made testing jig (Geraldini's jig) with a cyanoacrylate

glue (Model Repair II Blue, Dentsply-Sakin, Japan), and subjected to tensile load at a crosshead speed of 0.5 mm/min until failure (Instron 4411, Instron Corporation, Canton, MA, USA). All fractured surfaces were sputter-coated with gold/palladium and had their failure mode examined under scanning electron microscopy (JEOL-5600 LV, Tokyo, Japan) at 85 $\times$ magnification at 15 kV. The fracture modes were classified as: 1) cohesive failure in resin (CR), if the fracture occurred exclusively within the resin composite, adhesive, or both; 2) cohesive failure in dentin (CD); 3) interfacial failure if the fracture site was located entirely between the adhesive and dentin (I), 4) mixed failure (M), if the fracture site continued from the adhesive into either the resin composite or dentin. In case of uncertainty, higher magnifications (500–4000 $\times$) were used to confirm the nature of fracture. The number of prematurely debonded specimens per each tested group was also recorded but was not included in the statistical analysis to determine the final mean bond strength.

Statistical Analyses

Bond strength results of all actual tested specimens were analyzed by multiple ANOVA. In principle, normal (ND) and caries-affected (CAD) dentin substrates were separately analyzed by two three-way ANOVA analyses, having as main factors: the dentin pretreatment (control *vs* 2%CHX), adhesives (MP *vs* SB) and testing time (immediately *vs* 6 months after aging). Comparisons between the bond strength of ND *vs* CAD substrates at the baseline (immediately tested) or after 6 months of aging were then, individually, performed by two three-way ANOVA analysis, having the following main factors: dentin condition (ND *vs* CAD), dentin pretreatment (control *vs* 2% CHX) and adhesives (MP *vs* SB). Bonferroni's adjustment tests were used to examine *post hoc* differences among groups. ANOVA and Tukey's tests were also used to compare the effect of the pretreatment (control *vs* 2% CHX) on the distribution of failure modes either in normal or in caries-affected dentin. Statistical significance was preset at $\alpha=0.05$.

RESULTS

Results of bond strength reported as mean and standard deviation are summarized in Figures 1 and 2. CHX pretreatment did not affect the *in vitro* bond strength of specimens tested at immediate period (i.e. right after beams' preparation – baseline results) ($p>0.05$), regardless of the dentin condition (normal or caries-affected dentin) or the tested adhesive (MP or SB) (Figure 1). In general, 6-month storage in artificial saliva resulted in a significant reduction of bond strength for control and CHX-pretreated groups that were bonded with the more simplified tested adhesive, the two-step etch-and-rinse system Single Bond 2 ($p<0.05$) (Figure 1). The only exception were the specimens of caries-affected dentin pretreated with CHX, for which the bond strength of SB was not altered over the six months of storage in artificial saliva ($p>0.05$) (Figure 1B). In contrast, the bond strength of specimens bonded with the three-step MP system did not vary significantly over the six months of storage ($p>0.05$) (Figure 1), except for the control specimens of normal dentin that exhibited significant decrease of bond strength at this testing period ($p<0.05$) (Figure 1A). Immediate bond strengths (baseline results) of both tested adhesives to normal dentin were significantly higher than to caries-affected dentin ($p<0.05$) (Figure 2A). At this period, differences in bond strength by using SB or MP were observed for caries-affected dentin pre-treated with CHX, with SB giving higher bond strength than MP ($p<0.05$) (Figure 2A). After 6-month storage, differences between the bond strength to normal and caries-affected dentin were not significant ($p>0.05$), irrespective of the dentin treatment and tested adhesive (Figure 2B). At this period, control specimens bonded with MP exhibited higher bond strength when compared to control specimens bonded with SB ($p<0.05$) (Figure 2B). For all tested groups and conditions, the most prevalent fracture pattern observed was the mixed failure, following by interfacial failure (Table 2). Cohesive failures in resin were more prevalent for

specimens of normal dentin, while the prevalence of cohesive failure in dentin was greater for caries-affected specimens (Table 2 and Figure 3).

DISCUSSION

Since the application of 2% CHX did not affect the immediate bond strength of the tested etch-and-rinse adhesives to normal or caries-affected dentin, the primary tested hypothesis is supported. Nevertheless, the results showed that preservation of bond strength to normal dentin over the six months of storage in artificial saliva was significantly dependent on the adhesive and the application of 2% CHX, resulting in the partial rejection of the secondary tested hypothesis. This finding supports the concept that the use of chlorhexidine, as a therapeutic primer, may produce hybrid layers within normal dentin that, at least at middle-term (i.e. 6 months), would be mechanically more stable than untreated caries-affected dentin. Since all tested specimens were stored in the same artificial saliva containing the antimicrobial agent (i.e. sodium azide) that was replaced weekly, the possibility of bacteria being responsible for the loss of bond strength in this study is minimal. The artificial saliva always remained very clear indicating that no microbial growth occurred.

In addition to its well-known disinfectant/antimicrobial benefits, CHX has been examined as a therapeutic primer on the preservation of hybrid layers in human dentin using etch-and-rinse adhesives.^{30–32} While the antimicrobial efficiency of CHX on acid-etched dentin remains unclear, the present data confirms its positive effects on the preservation of bond strength when applied to demineralized normal dentin prior to bonding with no rinsing. CHX applied on exposed collagen fibrils and then sealed in place with adhesive resin/resin composites may protect the collagen against a dentinal collagenolytic attack, thereby delaying the typical degradation seen when using contemporary simplified dental adhesives.^{30–32}

CHX is an effective synthetic MMP-inhibitor that even in low concentrations (0.02 – 0.0001%) could directly inhibit the activity of MMP-2, -8 and -9.³⁴ These are the same MMPs that have recently been found in human dentin.^{24–26} At low concentrations, the inhibitory effect of CHX on MMPs is thought to be related to a cation-chelating mechanism, wherein the sequestration of metal ions, such as calcium and zinc, would hamper the activation of the catalytic domains within MMPs.^{35,36} Nevertheless, the MMP inhibitory effect of CHX seems to be dose-dependent³⁴, and at high concentrations it may likely inactivate MMPs by enzyme denaturation³⁷ rather than by chelation of cations.

In this context, reduction of bond strength in CHX-treated dentin would be more dependent on the degradation of hydrophilic polymerized adhesives. In fact, the present results did also show that, regardless of the dentin condition (normal or caries-affected), reductions in bond strength over six months were significantly greater for specimens bonded with the simplified two-step etch-and-rinse system - Single Bond 2 when compared to that observed for specimens that were bonded with the conventional three-step etch-and-rinse system - Scotchbond Multi Purpose (Figure 1). Although none of the current adhesive systems yet appears to be able to guarantee perfect-sealed, leakage-free restorations for a significant period of time¹¹, good clinical performance of the three-step etch-and-rinse adhesives over the simplified etch-and-rinse adhesives is notable.^{38,39} The presence of a relatively more hydrophobic, non-solvated resin over the hybridized dentin when using the three-step etch-and-rinse adhesives may improve the quality of the dentinal sealing by decreasing the permeability of the resin-bonded interface.^{14,40–42}

If adhesive resins can seal the acid-etched dentin from water, they will have their own mechanical/chemical properties preserved and, at the same time, they will protect collagen

against a hydrolytic potential of endogenous MMPs. MMPs are enzymes that require water to hydrolyze peptide bonds in the collagen molecules²⁹. Thus, besides being important to guarantee the integrity of the resin components of hybrid layers⁴³, the lower permeability of hydrophobic adhesives may have an additional effect on the durability of the resin-dentin interfaces by preventing an earlier exposition of dentin matrix to water and, then, to hydrolysis via dentinal MMPs.

Since carious dentin has been reported to exhibit increased collagenolytic activity when compared to intact normal dentin⁴⁴, it was previously considered that degradation of caries-affected dentin-bonded interfaces by dentinal collagenases may occur much more rapidly than in resin-bonded interfaces made in normal, noncarious dentin.³⁰ Interestingly, most of the presently tested caries-affected dentin-bonded specimens did not exhibit a significant reduction of bond strength over the six months of storage in artificial saliva, with the exception of the control group bonded with Single Bond 2 (Figure 1B). Under the scope of a collagenolytic process, a possible explanation for such results would be that the concentration of collagenases bound to collagen fibrils in caries-affected dentin may be relatively low. Nevertheless, the significant increase in the rate of cohesive failure in bonded caries-affected dentin accompanied by a higher number of premature debonding failures (Table 2) indicate that the mechanical stability of caries-affected resin-dentin bonds must be carefully interpreted.

As reported in previous studies^{4,5,45}, the caries-affected/adhesive interfaces exhibited significantly lower bond strength values than normal dentin/adhesive interfaces, regardless of the adhesive (i.e. MP or SB) and the dentin treatment (i.e. control or CHX) (Figure 2). Additionally, in comparison to noncarious dentin, the caries-affected dentin forms resin-bonded interfaces that are more complex, being composed of multiple zones (i.e. resin-infiltrated dentin, poorly-infiltrated dentin, exposed dentin and partially demineralized dentin).⁶ The presence of these different zones, with different stiffness/flexibility, raises concern whether this variable structure will be able to act as a strong foundation to dissipate stress from resin polymerization shrinkage, occlusal loading and thermal dimensional changes.⁴⁶ The softer the caries-affected dentin, the lower is its ultimate tensile or cohesive strength.⁹ It is this lower cohesive strength that might be responsible for the higher incidence of cohesive failures in caries-affected *versus* normal dentin (Table 2).

In conclusion, for optimal durability of resin-dentin bonds, preservation of resin and dentin matrix components should be equally considered. Although the present results do not prove that CHX can directly inhibit the collagenolytic activity of dentin, it is encouraging that CHX-treatment has once more demonstrated its therapeutic action on the preservation of the bond strength of etch-and-rinse adhesives to normal dentin. While the present protocol requires additional confirmation, further studies should also test the applicability of CHX and other synthetic MMPs inhibitors as therapeutic primers combined with the use of hydrophobic dental adhesives applied to acid-etched dentin saturated with ethanol instead of water.⁴⁷

Acknowledgments

This study was performed by Paula Carolina Komori as partial fulfillment of her M.S. degree at the University of Campinas. The authors gratefully acknowledge the valuable assistance given by Ms. Michelle Barnes for her editorial support and by Mr. Marcos Cangiani for his laboratorial support. The adhesives used in this study were kindly donated by 3M/ESPE. This study was supported by grants: 473164/2007-8 and 300615/2007-8 from CNPq (P.I. Marcela Carrilho), 111724 from the Academy of Finland (P.I. Leo Tjäderhane) and R01 DE014911 from the NIDCR (P.I. David H. Pashley).

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CLINICAL RELEVANCE

Attempts to use CHX adjunctively with conventional etch-and-rinse adhesives may play a clinically relevant role on the preservation of resin-dentin bonds.

Figure 1.

Bond strengths (MPa) to normal (Fig 1.A) and caries-affected (Fig 1.B) dentin in either control (Control) or CHX-pretreated (2% CHX) specimens that were tested immediately (MP baseline and SB baseline) or after six months of aging in artificial saliva (MP 6 mon. and SB 6 mon.). MP= Scotchbond Multi-purpose adhesive; SB= Single Bond 2 adhesive. The height of the bars is the bond strength mean; half-brackets indicate plus one standard deviation. Groups identified with different letters were statistically different ($p < 0.05$).

Figure 2.

Bond strengths (MPa) to normal (ND) *versus* caries-affected (CAD) dentin for control (**Control**) and CHX-pretreated (**2% CHX**) specimens that were tested immediately (Fig 2.A) or after six months of aging in artificial saliva (Fig 2.B). **MP**= Scotchbond Multi-purpose adhesive; **SB**= Single Bond 2 adhesive. The height of the bars is the bond strength mean; half-brackets indicate plus one standard deviation. Groups identified with different letters were statistically different ($p < 0.05$).

Figure 3.

Representative scanning electron micrographs (SEM) of fractured specimens composed of caries-affected dentin that was pretreated or not with chlorhexidine (**Control** or **CHX**) and submitted to tension **immediately** or after 6 months of storage in artificial saliva (**6 months**). A1) Low-power magnification (100×) demonstrates a mixed failure of a control specimen immediately tested; A2) Higher magnification (400×) of the area limited by a rectangle in A1, showing cohesive failures in the adhesive (A), resin composite (RC) and the bottom of the hybrid layer (BHL). B1) Low-power magnification (100×) demonstrates a mixed failure of a CHX-pretreated specimen immediately tested; B2) Higher magnification (300×) of the area limited by a rectangle in B1, showing cohesive failures in the adhesive (A), resin composite (RC), bottom of the hybrid layer (BHL) and dentin (D). C1) Low-power magnification (100×) demonstrates a failure almost exclusively in dentin (D) for a control specimen tested after 6 months of storage in artificial saliva; C2) Higher magnification (600×) of the area limited by a rectangle in C1, showing a large cohesive failure in the dentin (D) and a restricted cohesive failure at the bottom of the hybrid layer (BHL). D1) Low-power magnification (100×) demonstrates a mixed failure of a CHX-pretreated specimen tested after 6 months of storage in artificial saliva; D2) Higher magnification (500×) of the area limited by a rectangle in D1, showing cohesive failures in the adhesive (A) and dentin (D). **RC**= resin composite; **A**= adhesive; **D**= dentin; **BHL**= bottom of hybrid layer; **THL**= top of hybrid layer.

Table 1

Components of the artificial saliva used for the storage of the specimens.

Component	Concentration (mmol/L)
CaCl ₂	0.70
MgCl ₂ ·6H ₂ O	0.20
KH ₂ PO ₄	4.00
KCl	30.0
NaN ₃	0.30
HEPES buffer (acid)	20.0

Based on Pashley *et al.* (2004) - *J Dent Res* 83:216–221.

Table 2

Distribution of failure modes (in percentage) between groups, as observed with SEM.

Group	Pretreat	Test	Fract/Deb	Percentage of failure mode of fractured bonds (%)			
				CR	CD	I	M
MP/ND	C	IM	36/0	25.0 ^{bA}	0.0 ^{cB}	25.0 ^{bA}	50.0 ^{aC}
	CHX		30/0	0.0 ^{cD}	0.0 ^{cB}	25.0 ^{bA}	75.0 ^{aA}
	C	6 mon.	48/2	6.6 ^{cC}	3.3 ^{dA}	16.6 ^{bB}	66.6 ^{aB}
	CHX		40/1	11.1 ^{cB}	0.0 ^{dB}	22.2 ^{bA}	66.6 ^{aB}
MP/CAD	C	IM	20/1	0.0 ^d	10.0 ^{cB}	20.0 ^{bB}	70.0 ^{aB}
	CHX		19/1	0.0 ^c	11.0 ^{bB}	10.8 ^{bC}	78.2 ^{aA}
	C	6 mon.	31/9	0.0 ^d	20.8 ^{bA}	7.0 ^{cD}	68.7 ^{aB}
	CHX		28/5	0.0 ^d	20.0 ^{bA}	12.2 ^{cA}	67.8 ^{aB}
SB/ND	C	IM	29/0	10.3 ^{cB}	3.4 ^{dB}	17.2 ^{bA}	69.1 ^{aB}
	CHX		32/0	14.4 ^{bA}	6.5 ^{dA}	10.4 ^{cB}	68.7 ^{aB}
	C	6 mon.	43/1	4.6 ^{cC}	0.0 ^{dC}	16.3 ^{bA}	79.0 ^{aA}
	CHX		48/1	16.6 ^{bA}	0.0 ^{fC}	12.5 ^{cB}	70.8 ^{aB}
SB/CAD	C	IM	22/2	0.0 ^d	9.0 ^{cB}	24.2 ^{bA}	66.6 ^{aB}
	CHX		25/1	0.0 ^d	11.8 ^{cB}	17.6 ^{bC}	70.6 ^{aA}
	C	6 mon.	33/9	0.0 ^c	20.6 ^{bA}	19.7 ^{bBC}	59.1 ^{aC}
	CHX		34/6	0.0 ^c	17.0 ^{cA}	22.0 ^{bAB}	61.0 ^{aC}

MP: Scotchbond Multi-purpose system; **SB:** Single Bond 2 system; **ND:** normal dentin; **CAD:** caries-affected dentin; **C:** control dentin pretreatment; **CHX:** chlorhexidine dentin pretreatment. **IM:** values obtained at the immediate testing period; **6 mon.:** values obtained after 6 months of aging; **Fract/Deb.:** total number of fractured/debonded specimens (i.e. that failed before the test). **CR:** cohesive failure in resin; **CD:** cohesive failure in dentin; **I:** interfacial failure; **M:** mixed failure. Values are the percentages of failure modes in fractured specimen under the same type of dentin and adhesive (grouped and identified with the same shading background). Analyses comparing different types of dentin and/or adhesives were not performed. Different superscript lower case letters (analysis in row) and different superscript upper case letters (in column) indicate statistically significant differences ($p < 0.05$) (ANOVA and Tukey's tests).