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SEM evaluation of resin-cariou dentin interfaces formed by two dentin adhesive systems

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Abstract

Objectives—We investigated the influence of dentin tubule direction and identifiable zone of carious dentin on the microstructure and the thickness of the hybrid-like layer (HL) formed by self-etch and etch-rinse adhesive systems.

Methods—An etch-rinse and a self-etching adhesive were bonded to dentin carious zones divided into groups with parallel or perpendicular orientation relative to the dentin tubules at the resin-cariou dentin interface (N = 5/variable). Bonds were prepared to each of the four zones of carious dentin apparent after staining with Caries Detector: pink, light pink, transparent and apparently normal Six non-cariou third molars were controls. The microstructure and thickness of the HL were determined by SEM. and compared using three-way ANOVA and Tukey's multiple comparisons (p<0.05).

Results—Etch-rinse controls gave thicker HLs than self-etching systems; ; orientation did not affect thickness for the self-etch system. Perpendicular orientations gave thicker HLs than parallel for the pink zone bonded with the etch-rinse system. For both adhesives, HL thickness in the pink zone were significantly greater than in light pink for the perpendicular group, but no significant differences were found among other variables. HL microstructure was more granular and rougher for the etch rinse than for the self etching system. Pores and cracks were obvious in the more demineralized zones, Resin tags were shorter and irregular in the transparent zone and often were completely absent in the outer demineralized zones (pink, light pink).

Significance—Microstructure of bonded interfaces varies markedly depending on adhesive system, tubule orientation and carious zone.

Keywords

dentin bonding interfaces; dentin caries; microstructure; SEM; hybrid layer thickness

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Introduction

The primary aim of contemporary restorative dentistry in the excavation of carious dentin is to remove only the outer layer of carious dentin which is infected and is considered to be non-remineralizable. However, clinical guidelines for removal of this substrate are problematic and controversial. According to previous studies, the inner affected layer of carious dentin which is un-infected and remineralizable can be softer than normal dentin [1,2] or have similar properties to normal dentin. [3] These findings suggest that traditional tactile based characteristics of carious dentin often sacrifice more structure than necessary. Additionally, it is possible that clinicians are bonding to a substrate that is composed of sound, caries-affected and caries-infected dentin in the same cavity, which is likely to result from subjectivity inherent among dentists and the variation in the quality and quantity of dentin removed during treatment [4]. Using caries detector may be helpful to differentiate the layers of carious coronal dentin, by staining the outer layer pink [5]. Although it has been criticized as being insufficiently discriminating [6], it is clear, based on degree of staining, that the carious dentin lesion can be classified into pink, light pink, transparent, and apparently normal zones [3]. These four zones have distinct mechanical properties, which may guide clinical decisions to excavate or remineralize carious dentin.

Recent studies showed that the application of adhesive-sealed composite restorations to irreversible infected dentin did not affect the clinical performance of those restorations [7]. With the advent of contemporary antibacterial adhesive systems, it may be possible to disinfect cavities containing residual bacteria using adhesives with strong antibacterial effect. [8]. Studies have shown that an antibacterial monomer, 12-methacryloyloxydodecyl-pyridinium bromide (MDPB), has strong antibacterial activity against oral bacteria and exhibits inhibition of bacterial growth after incorporating it into the polymer network of the cured resin [9]. It may be useful in eliminating harmful effects caused by bacteria, as well as contributing to better prognosis for minimal restorative treatments of dentin caries. A bond between adhesive resin and dentin depends on the penetration of the primer and adhesive resin into the conditioned dentin surface to create micromechanical interlocking between the dentin collagen and resin, forming a hybrid layer [10]. The formation of the hybrid layer may be affected by chemical composition and application mode of adhesive systems [11], differences in the dentin location [12,13] and changes in the microstructural features of dentin associated with the caries process [14]. Several previous studies have shown that the hybrid layer created by etch-rinse systems is significantly thicker than that created by self-etch primers [14–16] since they are more acidic and therefore result in a deeper demineralized layer. This permits deeper penetration of adhesive resin during bonding. There were no consistent results regarding the influence of direction of dentin tubules on the thickness of the hybrid layer. Harnitattisai *et al.* described the hybrid layer as thinnest at the lateral walls of the cavity surface [12]. In contrast, the work of Ogata *et al.* [17] indicated the hybrid layer created on the surfaces parallel to the tubules had significantly greater thickness than those in the perpendicular group. The hybrid layer for specimens bonded perpendicular to the tubule axis appeared to be more uniform than that for specimens bonded parallel to the tubules [13,17], but there was no difference in the thickness of the hybrid layer between the specimens with different tubule orientations.

Carious dentin has a Knoop hardness that is less than that of normal dentin [1,15], indicating that it has a certain level of demineralization, even though the lumina of many of the dentin tubules are occluded with mineral crystals [18] to form the transparent layer. Dentin that is partially demineralized by caries may exhibit further mineral loss when it is treated with acidic bonding conditioners, resulting in a deeper penetration of adhesive resin and a thicker resin infiltrated layer formation during bonding.

The aim of this research was to compare the microstructure of the adhesive interface of two dentin bonding systems: Single Bond Plus (3M/ESPE, St Paul, MN) (an etch-rinse system) and Clearfil SE bond (Kuraray America, New York, NY) (a self-etch system) at various zones of carious dentin and with two dentin tubule orientations, using optical and scanning electron microscopy (SEM). Thus the hypothesis tested was that there are important differences in microstructure of the interfaces that depend on the zones of the carious lesion, tubule orientation and the nature of the bonding system (etch-rinse or self-etching).

MATERIALS & METHODS

All subjects enrolled in this research provided informed consent for the protocol, which had been approved by the UCSF Institutional Committee on Human Research. The extracted teeth, which contained no prior restorations, were sterilized by gamma radiation and stored in Hank's balanced salt solution at 4°C [19]. In the parallel group, 5 third molars with coronal dentin caries were prepared using the following procedures (Fig. 1): after longitudinally sectioning a tooth through the center of its lesion using a water-cooled saw (Isomet Low Speed Saw; Buehler Ltd., Lake Bluff, IL, USA) with a 0.15 mm thick diamond blade, the entire cut surface was flooded with Caries Detector solution to stain the lesion (Kuraray Medical Inc., Tokyo, Japan). Four distinct zones were identified in the lesions based on the degree of staining: pink, light pink, transparent and an apparently normal zone, as characterized by Zheng *et al.* [2]. The surfaces, with tubules approximately parallel to the surface, were finished with 320 grit SiC paper under running water. In addition, 3 third molars without caries were prepared as described above for the control parallel group.

For the perpendicular group, 5 carious third molars were prepared by the following procedures (Fig. 1): the occlusal surfaces were wet ground perpendicular to the long axis of the teeth to expose a flat surface where each caries lesion was surrounded by normal dentin. In this case, the exposed surface was approximately perpendicular to the dentin tubules. The specimens were ground under running water using the degree of Caries Detector staining as a guide, to expose the four zones of carious dentin. However, it was not possible to expose all four zones in the same plane, so teeth with carious dentin exhibiting light pink, transparent, and apparently normal zones were selected. In addition, another five molars were prepared in the same way to expose the pink zone of carious dentin. Each of these specimens were then cut vertically through the middle of the lesion, forming two halves and the designated bonding surfaces were finished as described before. The remaining dentin thickness (RDT) of each specimen in the perpendicular group was measured as the distance from the center of the bonded area to the closed region of the pulp chamber after specimen preparation. This RDT-value was used to prepare another 3 third molars without caries to expose dentin at approximately the same RDT for the control perpendicular group.

In all four groups, one half of each tooth was bonded with a self-etch system, Clearfil SE Bond (SE, Kuraray-America, New York, NY) according to the manufacturer's instructions. The prepared surface was treated with the acidic primer for 20 seconds, and dried with a stream of air; the adhesive was applied; and the specimen was air dried and light-cured for 10 seconds. An etch-rinse adhesive, Single Bond Plus (SB, 3M/ESPE, St Paul, MN), was applied to the other half according to the manufacturer's instructions. The tooth specimen was etched for 15 seconds with 35% phosphoric acid, washed with water for 10 seconds and blot-dried. Two coats of adhesive were applied, gently blow-dried for 5 seconds and light-cured for 10 seconds. After the bonding process, composite build-up was performed with a hybrid resin-based composite (Z250 Restorative, 3M/ESPE; St Paul, MN) in two 1.5 mm-thick increments. The specimens were then stored in water at 37°C for 24 hrs. The bonded specimens of the parallel and control parallel groups were sectioned longitudinally and perpendicularly to the bonded surface to expose the resin/dentin interface, and the specimens in all groups were polished,

sequentially, using waterproof silicon carbide papers under running water with grit sizes of 600, 800, and 1200 and diamond pastes of 3, 1, and 0.25 μm . Each polished surface was stained by Caries Detector again to identify each zone. In the parallel and perpendicular groups, fiduciary marks were made at the composite side near the interface between different zones with a #11 scalpel under 40X optical microscopy. In the control parallel group, fiduciary marks were made from the pulp chamber at 1 mm intervals at the composite side near the interface. Immediately after identification of carious zones in the lesion, all specimens were prepared for SEM analysis. They were fixed in 3% gluteraldehyde in a 0.1 M sodium cacodylate buffer (pH=7.4) for 12 hrs at 4°C, rinsed with 0.2 M sodium cacodylate for one hour in three different baths (20 mins each), and rinsed for one minute with deionized water. The specimens were subjected to 0.1 M hydrochloric acid treatment for 90 seconds, followed by immersion in 1% sodium hypochlorite for 10 mins to reveal the hybrid-like layer. The specimens were then dehydrated in ascending grades of ethanol to 100%, transferred to HMDS and allowed to air-dry for 10 mins [20]. Finally the specimens were sputter-coated with 200 nm gold (SCD050 Sputter Coater; BAL-TEC, PA, USA). One SEM image at 3000X was taken of the resin/dentin interface in the middle area of each zone for measurements. Additional images at 10,000X were taken to illustrate important features. In the parallel and control parallel groups, because of lack of resin tags, the thickness of the hybrid-like layer was measured as the distance between the top and the bottom of the layer. In the perpendicular and control perpendicular groups, layer thickness was determined as the distance between the top of the hybrid layer and the height of the scalloped convexities at the base of the hybrid layer between adjacent resin tags. There were 5 measurements of width of hybrid-like layer in each SEM image that were made using image analysis software (NIH image, <http://rsb.nih.gov/ii/>). Three measurements were made at approximately equally-spaced points across the image and two measurements representing the narrowest and widest thicknesses in the image were also recorded.

Statistical analysis

The mean and standard deviation of the thickness of the hybrid-like layers were calculated for each zone of each group. The thicknesses of the layers for various caries zones for each bonding system and tubule orientation were compared using 3-Way ANOVA (factors: material, orientation, caries zone) and additional one-way AVOVAs. The thicknesses for the two tubule orientations for each bonding system and caries zone and the thicknesses for the two bonding systems for each tubule orientation and carious zone were compared using Student's t-tests.

Results

The thickness of the hybrid-like layer of each group is given in Table 1. In the control groups of each adhesive system with RDT of 2 mm, the tubule orientation did not have an effect, and as expected, the hybrid layer was thicker for the etch rinse than for the self-etch system. However, for the etch-rinse parallel groups, RDT thickness of 2 mm yielded significantly thicker layers than those at 3 mm.

Scanning electron micrographs of the polished cross-sections of the bonded specimens of each tubule orientation and each zone of carious dentin are shown in Fig. 2 and Fig 3. For the specimens of the perpendicular group using the etch-rinse system (Fig. 2), a hybrid layer could be clearly observed in all 4 zones of carious dentin and appeared to be more granular than those of specimens bonded with the self-etch system (Fig. 3). In the transparent zone, some pores and cracks were found in the middle and/or base of the hybrid-like layer. There were more obvious pores and cracks in the light pink and pink zones. In the light pink and pink zones for both adhesive systems; separation between composite and adhesive layer was common and was a result of shrinkage during the specimen preparation for SEM. In some specimens, this shrinkage caused cohesive fracture in the adhesive layer and/or in the highly demineralized

carious dentin. The resin tags found for apparently normal dentin in specimens of the perpendicular group using etch-rinse adhesive were funnel-shaped due to the dissolution of peritubular dentin in the top 3 to 4 μm around the dentin tubules (Fig. 2-a). There were lateral extensions of resin tags into the lateral branches of the tubules of apparently normal dentin. In the transparent zone, the resin tags were shorter and more irregular in shape (Fig. 2-c). There were few lateral extensions of resin in this zone. Resin tags were uncommon and much more variable in shape in the pink zone (Fig. 2-g). They were thin, irregular, highly porous and easily broken during specimen preparation. Observation of the specimens of the parallel group using the etch-rinse system showed that resin tags run parallel to the resin-dentin interface with lateral branches beneath the hybrid layer. In some dentin locations, the hybrid layer was created not only under the bonded interface but also beneath the resin tags (Fig. 2-f). In some cases this made it difficult to distinguish between the hybrid-like layer and a tag in close proximity. Compared to the specimens of the perpendicular group, there seemed to be more obvious separation and porosity in the hybrid-like layer at each zone of the carious lesion in the parallel group.

For the specimens bonded perpendicular to the tubules using the self-etch system, the hybrid-like layer appeared to be very thin and uniform (Fig. 3-a). The hybrid layer was difficult to discern at some locations in the apparently normal dentin. In this zone, resin tags were cylindrical and slender with very thin lateral extensions into the lateral branches of tubules. The appearance and texture of the hybrid-like layer was smoother and more intact for the self-etch as compared to the specimens bonded with etch-rinse system. There were fewer resin tags found in the other 3 zones and they were shorter and more porous as the level of demineralization increased. Separation in for the self-etch system was mainly found between adhesive layer and composite without the relatively common fractures in the adhesive which were observed for the etch-rinse system. (Fig. 3-g, h). Cohesive fracture in highly demineralized infiltrated dentin was sometimes evident. Both adhesives exhibited different adhesive interface microstructures among different zones of carious lesion.

The thicknesses of the hybrid-like layers for the various zones for each system in the two orientations are shown in Table 1. An analysis of variance revealed statistically significant interactions among orientation of dentin tubules and the caries zones. To assist in interpreting this interaction, the mean difference of the layer thicknesses was computed for each zone of carious dentin in both adhesive materials (Table 2). These data indicated the thickness of the layer in the pink zone was significantly greater than that in the light pink zone when specimens were bonded perpendicular to the dentin tubules for both adhesive systems. However, there was no statistically significant difference among the light pink zone and the other two zones, nor was there a difference among all 4 zones of specimens bonded parallel to the dentin tubules. The layer for the etch rinse system in the pink zone of the perpendicular group was greater than in the parallel group ($p < 0.05$). There was no statistically significant difference ($p > 0.05$) in the other three zones of carious dentin. In the self-etch groups, there was no significant thickness difference between parallel and perpendicular bonded groups.

Comparison of the thickness for the two adhesive systems in the perpendicular group showed that the etch-rinse produced thicker layers than the self-etch system ($p < 0.01$) at light pink, transparent and the apparently normal dentin zones. In the pink zone, there was no significant difference between the thicknesses for these two adhesive systems, but the standard deviations were very large. In the parallel group, the thickness using etch-rinse was also significantly greater than for the self-etch system in the transparent zone and apparently normal dentin ($p < 0.05$). However, there was no significant difference in the pink and light pink zones.

Thus, we accept part of our hypothesis that hybrid-like layers of the most demineralized carious zone are thicker than those in apparently normal or transparent dentin.

Discussion

A previous study showed that the sealing ability of a self-etch primer on caries-infected dentin was poorer than to caries-affected dentin and normal dentin after a period of time [21]. This might be due to increased water content, porosity and permeability of caries-infected dentin because of reduced dentin mineralization. As mineral is removed the dentin matrix takes up additional moisture and when bonded with hydrophilic bonding agents any hydrolytic instability would increase. As Sano [22] described, such zones stain pink because of high porosity. In the demineralized dentin matrix, resin monomer infiltration yielded thicker hybrid-like layers, which appeared to be porous and of poorer quality than in normal dentin. The increased water may dilute the organic solvents of some bonding systems, causing monomers to leave the soluble phase and form resin globules in water [23]. In addition, the conversion of monomers to polymers might be impeded. In agreement with previous studies [21,24], the hybrid layer of the pink zone was the thickest of all 4 zones with much substrate variation and a wide range of 10 to 50 μm . This high variance indicated the unpredictability of the demineralization process in carious lesions. Van Meerbeek *et al.* [11] demonstrated that, in some dentin bonding systems, there was a discrepancy between the depth of dentin demineralization and the depth of resin infiltration. In our investigation, this discrepancy was more obvious in the pink zone bonded with the etch-rinse system. Parts of the phosphoric acid-etched dentin surface of some specimens were not completely wetted by resin, resulting in a band of disorganized collagen fibrils approximately 10 to 15 μm thick found beneath the resin-infiltrated demineralized zone (Fig. 2-h).

Some of our specimens treated with 35% phosphoric acid showed cohesive fracture of the resin infiltrated pink zone, presumably due to the reduced mechanical properties of the more highly demineralized zones [2]. Bond strength to caries infected dentin was about 10 MPa in Yoshiyama's study, less than one-fourth of the bonding strength of normal dentin. [24]. The other reason for the fracture in this caries-degraded dentin might be that the collagenous matrix was further weakened by depletion of mineral and degradation of proteins by the acidity of phosphoric acid or the action of proteases.

Previous studies reported thinner hybrid layers in superficial dentin compared with deeper dentin [25,26]. This is in agreement with the results of the control parallel group for the etch-rinse system in our study; the thickness of the hybrid layer was significantly greater ($p<0.05$) for smaller RDT, closer to the pulp chamber. However, RDT had no effect on the thickness of the hybrid layers in the self-etch parallel group, which were much thinner in all cases. The mild acidic components in self-etching primers demineralize through the smear layer and diffuse only a short distance into the underlying dentin, regardless of the differences between superficial and deep dentin. In addition, there was no significant difference in the thickness of the hybrid-like layer between parallel and perpendicular control groups at the same RDT (2 mm). The same finding was noted in each zone of the experimental groups of both bonding systems except in the pink zone of the perpendicular group using SB. This exception could be explained by the high variance of this porous demineralized carious dentin, which might influence the outcome of acid treatment and subsequent resin infiltration. Another reason might be that the specimens for the pink zone of the perpendicular group came from different teeth because of the difficulty of exposing all four zones in the same plane. Our results do not support those of Ogata *et al.* [17], who compared the thickness of the hybrid layer of four commercially available adhesive systems. They found the hybrid layers of specimens bonded parallel to the dentin tubules were thicker than those of the perpendicular group. This may be because they used deeper dentin for the parallel group.

Specimens of the parallel group may not be bonded exactly parallel to the orientation of the dentin tubules, owing to their sigmoid curvature. In this situation, the adhesive resin could

penetrate and diffuse not only from the exposed dentin surface, but also from the dentin tubules into the surrounding demineralized intertubular dentin. This may increase the thickness of the hybrid layer when compared to truly parallel bonded specimens. Furthermore, at the location of the resin-dentin interface parallel to the dentin tubules, the resin tags could run parallel and connect with the bottom of the hybrid layer without a discernible interface between them (Fig. 2-f, right side). This led to some uncertainty in the measurement of the hybrid layer thickness in some areas. This might increase the apparent thickness of the hybrid layer by inadvertently including the resin tags. The unique characteristics found only in the parallel group supported our hypothesis that there were important differences in microstructure of the resin-dentin interface between the specimens bonded to different orientations of dentin tubules.

In the apparently normal and transparent dentin zones, the hybrid layers created by the etch-rinse system (SB) were thicker than those created by the self-etching system (SE). However, there was no significant difference of the hybrid layer thickness created in the pink zone between these two adhesive systems for either the perpendicular or parallel groups. This can be explained by the increased permeability of the highly demineralized dentin, which allowed deep penetration even with weakly acidic self-etching primer. In the perpendicular group, the hybrid layer thickness of the light pink zone using etch-rinse greater than for the self-etch group. Although there was no significant difference in the parallel group, there was a tendency for SB to create thicker hybrid layers.

Conclusions

We found that in the most demineralized zone (pink) zone of carious lesions had hybrid-like layers that were thicker when the tubules were oriented perpendicular to the bonded interface for the etch-rinse system. The self-etch system for both tubule orientations and the etch-rinse system in the parallel orientation did not produce significant differences in the other carious zones. Generally the hybrid-like layers were thicker in the more demineralized zones and the layers were highly variable and yielded poor layers with pores and cracks in the pink zone.

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Method 1: parallel group

Method 2: perpendicular group

Figure 1.

Schematic illustration of carious lesion divided into 4 zones based on the staining with Caries Detector: pink, light pink, transparent and apparently normal. The flat dentin surface was prepared for parallel group with method 1. The bonded surfaces were approximately parallel to the orientation of dentin tubules. By method 2, the bonded surfaces of perpendicular group were approximately perpendicular to the dentin tubules.

Figure 2.
SB

Figure 3.
SE Bond

Table 1

Comparison of the thickness of the hybrid layer: mean $\pm$ SD (μm) of each group, by adhesive, orientation of dentin tubules and zone of carious dentin

Zone of caries dentin	Parallel Group (n=5)		Perpendicular Group (n=5)	
	SE Bond Adhesive	3M Single Bond Plus Adhesive	SE Bond Adhesive	3M Single Bond Plus Adhesive
Pink zone	8.5 $\pm$ 7.5	7.9 $\pm$ 3.6 ^a	22.4 $\pm$ 12.8 ^c	20.8 $\pm$ 6.3, ^{ac}
Light pink zone	3.5 $\pm$ 1.8	7.2 $\pm$ 3.5	3.7 $\pm$ 1.8, ^c	8.6 $\pm$ 2.5 ^c
Transparent zone	1.6 $\pm$ 0.5 ^b	4.1 $\pm$ 0.7 ^b	1.5 $\pm$ 0.8, ^b	5.1 $\pm$ 0.9 ^b
Apparent normal dentin zone	1.0 $\pm$ 0.4 ^b	3.5 $\pm$ 0.6 ^b	0.8 $\pm$ 0.3 ^b	3.7 $\pm$ 0.7 ^b
B,d,f				
Remaining Dentin Thickness (RDT) of Normal dentin	Controls		Perpendicular Group (n=3)	
	SE Bond	3M Single Bond Plus	SE Bond	3M Single Bond Plus
1 mm	0.8 $\pm$ 0.2 ^b	5.0 $\pm$ 0.1, ^b	NA	NA
2 mm	0.6 $\pm$ 0.1 ^b	4.6 $\pm$ 0.2 ^{b,c}	0.6 $\pm$ 0.1 ^b	4.5 $\pm$ 0.6 ^b
3 mm	0.6 $\pm$ 0.2 ^b	3.5 $\pm$ 0.8, ^{bc}	NA	NA

^a Mean values are significantly different ($p<0.05$) when the thickness of the hybrid layer created in perpendicular and parallel specimens are compared within the same bonding system.

^b Mean values are significantly different ($p<0.01$) when the thickness of the hybrid layer created in 3M Single Bond Plus and Clearfil SE Bond are compared within the same orientation of dentin tubules and zone of carious dentin.

^c Mean values are significantly different ($p<0.05$) when the thickness of the hybrid layer created in different location are compared with the same bonding system.

Table 2Mean (μm) difference of the thickness of the hybrid layer in specimens bonded perpendicular to the dentin tubules.

Adhesive	(I) Caries zone	(J) Zone compared	Mean Difference (I-J)	Sig.
3M Single Bond Plus	Pink	light pink	12.2476 ^(*)	<.001
		Transparent	15.6216 ^(*)	<.001
		Normal	17.0636 ^(*)	<.001
	light pink	pink	-12.2476 ^(*)	<.001
		transparent	3.3740	.429
		normal	4.8160	.159
	transparent	pink	-15.6216 ^(*)	<.001
		light pink	-3.3740	.429
		normal	1.4420	.908
	normal	pink	-17.0636 ^(*)	<.001
		light pink	-4.8160	.159
		transparent	-1.4420	.908
Clearfil SE Bond	Pink	light pink	18.6936 ^(*)	.002
		transparent	20.8668 ^(*)	.001
		normal	21.5340 ^(*)	<.000
	light pink	pink	-18.6936 ^(*)	.002
		transparent	2.1732	.951
		normal	2.8404	.899
	transparent	pink	-20.8668 ^(*)	.001
		light pink	-2.1732	.951
		normal	.6672	.998
	normal	pink	-21.5340 ^(*)	<.001
		light pink	-2.8404	.899
		transparent	-.6672	.998

^(*) The mean difference is significant at the .05 level.

For both adhesive systems, the thickness of the hybrid layer in the pink zone was significantly greater than that in the light pink zone when specimens bonded perpendicular to the dentin tubules ($p < 0.01$). However, there was no statistically significant difference among the light pink zone and the other two zones.