Histomorphologic Characterization of Noncarious and Caries-Affected Dentin/Adhesive Interfaces

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Purpose: **The purpose of this study was to compare the dentin/adhesive interfacial characteristics when bonding to noncarious as well as caries-affected dentin.**

Materials and Methods: **Seven extracted, unerupted, third molars were sectioned into halves. Artificial caries was created on one-half of each tooth, leaving the other half as a control. Dentin surfaces were treated with UNO adhesive according to the manufacturer's instructions for the wet-bonding technique and under environmental conditions present in the oral cavity. Dentin/adhesive interface sections of each half-tooth were stained with Goldner's trichrome, a classic bone stain, and examined using light microscopy. The width of exposed collagen was measured directly from photomicrographs, and adhesive penetration was analyzed qualitatively.**

Results: **The degree and extent to which the adhesive encapsulated the demineralized dentin matrix were reflected in the color difference in the stained sections with the noncarious dentin sections showing a degree of collagen encapsulation superior to that of the caries-affected dentin sections. The overall mean widths of exposed collagen were significantly ($p \leq .05$) greater at the caries-affected dentin/adhesive interface, 8.6 (1.7) μm, as compared with those at the noncarious dentin/adhesive interface, 6.0 (1.5) μm.**

Conclusions: **The morphologic characteristics of the caries-affected dentin/interface suggest an increase in the exposed collagen zone and a decrease in the quality of the adhesive infiltration when compared with noncarious dentin. The evidence suggests that dentin substrate characteristics have a significant effect on the dentin/adhesive interface structure.**

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COMPOSITE RESIN has increasingly gained acceptance as a core buildup material.[1,2] Its rapid set and polymerization allow for the convenience of a single-visit core placement and preparation.[3,4] In addition, tooth-colored core material can be used under all ceramic restorations allowing for superior esthetic results. Nevertheless, debonding of core buildups as well as clinical and/or radiographic evidence of a marginal gap between the tooth and buildup material have been reported as a common problem with composite buildups.[5-7]

A fundamental factor in the effectiveness of using composite as a core buildup material is its ability to adhere to the tooth structure. A core must be anchored firmly or bonded to the tooth structure to allow the residual tooth and the associated buildup to act as one unit. Otherwise, it offers no advantage over allowing the bulk of the final restoration to occupy the space.[8] The success of the final restoration is dependent on the performance of the underlying tooth structure and composite core, which, in turn, is dependent on the formation of a

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long-lasting, strong, and microleakage-resistant adhesive bond between the core material and tooth structure.

With extensively damaged teeth, dentin is the main substrate to which the composite core must bond. Current theories suggest that achieving an ideal bond to dentin occurs when the polymerizable resin completely impregnates the partially demineralized dentin thus encapsulating and reinforcing any collagen exposed by the etching process.[9] This dentin-resin interdiffusion zone between the dentin surface and adhesive is designated the "hybrid layer." The hybridization process is considered essential in order to achieve initial maximum bond strength and maintain bond strength over time.[10,11]

Despite significant enhancements in dentin bonding technology, such adhesion is not yet achieved. Studies on noncarious dentin have revealed that the hybrid layer lacks complete infiltration of the resin into the demineralized dentin zone,[12,13] leaving a zone of unprotected protein vulnerable to attack by the oral fluids and enzymes, which may lead to debonding of the buildup or possibly microleakage and recurrent caries. These studies used a histomorphologic technique in which Goldner's trichrome stain will react with exposed collagen that is not encased in adhesive resin.[12-14] This novel staining technique, which identifies collagenous protein at the light microscope level, provides a unique, clear representation of the extent and degree to which the adhesive resins envelop the collagen fibrils of the demineralized dentin matrix. The differences in the extent and type of staining provided by the histomorphologic technique have been validated with a complementary technology such as micro-Raman spectroscopy.[14]

Much of the understanding about dentin bonding and the factors that impact the bond formed at the dentin/adhesive interface has been based on studies completed on noncarious human dentin. Noncarious dentin is not, however, the substrate most frequently encountered in clinical dentistry.[15] Instead, dentists are restoring teeth with carious dentin. Carious dentin is characteristically described as consisting of infected and affected layers. The affected layer is generally not removed during treatment. Based on structural features, this layer is subdivided into the following: turbid or discolored layer, transparent zone, and subtransparent zone.[16] The transparent zone occupies the largest portion of the caries-affected dentin. Very interestingly, in contrast to noncarious dentin, acid-resistant mineral deposits frequently occlude the tubules within the transparent zone. Results from bond strength studies suggest that the characteristics of the dentin substrate directly impact the bond strength of the dentin/adhesive interface. For example, authors reported a nearly 40% drop in bond strength when bonding to caries-affected dentin as compared with noncarious dentin.[17]

These differences in bond strength may be related to the structural difference between noncarious and caries-affected dentin, which could influence the pattern of dentin demineralization, adhesive penetration and consequently, the bond at the dentin/adhesive interface. To date, there has been limited investigation of the effect of caries-affected dentin on the morphologic characteristics of the dentin/adhesive interface. The purpose of this study was to use the histomorphologic staining technique to compare the dentin/adhesive interfaces formed when a two-step adhesive system (conventional etchant, primer/adhesive combination) was applied to both caries-affected dentin and noncarious dentin.

Materials and Methods

After obtaining approval from the University of Missouri - Kansas City Adult Health Sciences Institutional Review Board, seven extracted, unerupted, human third molars were collected from oral surgeons practicing in the metropolitan area. Sample size was determined by a power analysis of preliminary data at $\alpha = 0.05$ and $1-\beta = 0.8$. The extracted teeth were stored in a solution of 0.9% normal saline and 0.002% sodium azide at 4°C.

The specimen-sectioning procedures are presented in Figure 1. The occlusal third of the tooth was removed using a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL). Each tooth was further sectioned buccolingually into halves. All specimens were covered with wax, leaving a 3 × 2-mm window of exposed sound dentin. Each half-tooth was randomly assigned to be placed in a vial containing either phosphate-buffered solution (pH 7) as control, or acidified buffered solution (pH 4.5) to create artificial caries as described by Itthagarun et al.[18] This step allows each tooth to act as its own control. Vials were placed on an orbital shaker at 100 rpm and stored at 37°C. After 10 days, the wax was removed and artificial soft caries was excavated with hand instrumentation (#EXC38/39 spoon excavator, Hu-Friedy, Chicago, IL). A smear layer was created by

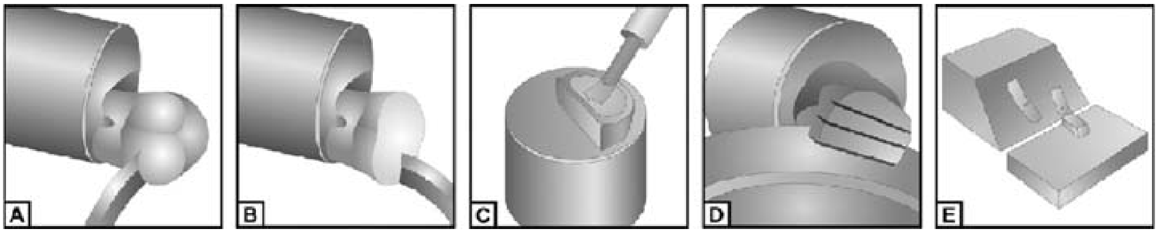

Figure 1. Schematic illustration of specimen preparation procedures: (*A*) The occlusal one-third of the crown removed. (*B*) The tooth sectioned bucco-lingually into halves. (*C*) A uniform smear layer created, followed by application of dental adhesive. (*D*) Parallel cuts made perpendicular to the adhesive interface, and a final cut made 2 mm below the interface. (*E*) 5 μm sections cut from the resulting slabs.

abrading the exposed dentin surface of all specimens with a 600-grit silicon carbide under copious water cooling.

The specimens representing caries-affected dentin and the normal noncarious dentin were treated with UNO adhesive (Lot no. 020105, Pulpdent, Watertown, MA) according to the manufacturer's instructions using a conventional etchant and the wet-bonding technique. UNO adhesive is a primer/bond agent combination that contains hydrophilic PMGDM (pyromellitic dianhydride with glycerol dimethacrylate) monomer and acetone solvent. To simulate the oral cavity conditions, adhesive application was performed in an environmental chamber at 37°C and 90% humidity. The adhesive application procedure was as follows: the dentin surface was etched with 38% phosphoric acid gel (Etch Rite, Pulpdent) for 15 seconds. To establish equal etching time for the entire dentin surface, the gel was dispersed on a small plastic slab. The slab was turned over and applied to the dentin surface for 15 seconds. After 15 seconds, the tooth was rinsed thoroughly with distilled water. The excess water was removed by blotting the surface gently (Task Wipes, Kimberly Clark Corp., Neenah, WI) until no pooling of water was visible. Every effort was made to avoid desiccation of the tooth surface.

Two coats of the adhesive were applied to the dentin surface with a small brush. Air was gently applied for 5 to 10 seconds after each coat. The adhesive was polymerized by a 30-second exposure to a visible light source (Spectrum light, Dentsply Caulk, Milford, DE). Dentin/adhesive specimens were stored for a minimum of 24 hours in a 0.96% phosphate-buffered solution (Dulbecco's PBS, Sigma Chemical Co, St Louis, MO) with 0.002% sodium azide at 37°C before proceeding with the sectioning.

Each treated half-tooth was sectioned perpendicular and parallel to the bonded surface by means of a water-cooled low-speed diamond saw (Buehler). A minimum of two resultant 8 × 2 × 2 mm slabs were obtained and then mounted on a methacrylate support with cyanoacrylate adhesive. One 5 μm-thick section was cut from the face of each slab by means of a tungsten carbide knife mounted on a Polycut S "sledge" microtome (Leica SM 2500S, Midland, Ontario, Canada). The resultant two sections were collected on glass microscope slides treated with Haupt's adhesive. This adhesive, a mixture of gelatin, glycerol, and phenol, was used to keep sections attached to the glass slide during the staining procedure. Differential staining was accomplished with Goldner trichrome, a classical bone stain.[19] Slides with adherent-stained sections were dehydrated through ascending ethanol and xylene. The sections were coverslipped and examined by light microscopy (Nikon Inc, Melville, NY) at 100× magnification.

With this staining technique, the exposed collagen stains a distinct red; collagen that is coated with adhesive stains orange; the mineral stains green; and pure adhesive appears beige.[12-14,20] A quantitative assessment of the width of the collagen available for staining was determined by direct measurement from photomicrographs whose exact magnification was established with a stage micrometer. The measurements were recorded using the software program analySIS® (Soft Imaging System Corp, Lakewood, CO). Five measurements, 30 μm apart, were made across both interface sections. Using separate sections and multiple measurements across each section provided a broader perspective of the dentin/adhesive interface throughout the specimen. Thus, for each half-tooth a total of ten measurements were made and averaged to obtain the mean exposed collagen depth for each specimen. An overall mean and SD were computed from the mean values for each treatment group and were statistically compared using a dependent t-test, $\alpha = 0.05$ (SPSS v.12, Chicago, IL).

Furthermore, a qualitative analysis of the quality of collagen encapsulation was completed by observing color differences within the interfacial zones of the caries-affected and noncarious dentin specimens.

Results

The quantitative data, mean measurements, overall means and SD of exposed collagen zones in noncarious and caries-affected dentin/adhesive

Table 1. Means, Overall Means, and Standard Deviations for the Exposed Collagen Zone in the Noncarious and Caries-Affected Dentin/Adhesive Interface Specimens

Specimen Number	*Noncarious Dentin Means ± SD (μm)*	*Caries-Affected Dentin Means ± SD (μm)*
1	6.4 (±1.5)	9.3 (±2.7)
2	3.6 (±0.7)	5.7 (±1.7)
3	8.1 (±0.8)	8.3 (±3.4)
4	7.1 (±1.0)	7.9 (±1.6)
5	5.5 (±0.6)	11.4 (±6.0)
6	4.7 (±0.9)	8.6 (±2.0)
7	6.6 (±1.6)	9.4 (±1.1)
Overall Mean and SD	6.0 (±1.5)	8.6 (±1.7)

specimens are presented in Table 1. The overall mean and SD for the exposed collagen zone for the noncarious dentin specimen was 6.0 (1.5) μm wide. The overall mean value for the width of exposed collagen in the caries-affected/dentin specimens was 8.6 (1.7) μm. The dependent *t*-statistical test revealed that the caries-affected dentin/adhesive specimens had significantly wider zones of exposed collagen ($p = 0.042$) than the noncarious dentin/adhesive specimens.

The qualitative analysis was based on the evaluation of photomicrographs. A representative light micrograph of a noncarious dentin/adhesive interface is presented in Figure 2. The photomicrograph shows a distinct color between the pure UNO adhesive (beige) and the mineralized dentin (green). The red-orange zone represents the zone of adhesive infiltration and exposed collagen. The orange color seen within this zone represents regions where the collagen was fully encapsulated by the UNO adhesive, i.e., hybrid layer, leaving no exposed protein available to react with Goldner's stain. The red color (~5 to 6 μm wide throughout Fig 2) represents collagen that was partially infiltrated with adhesive but not fully encapsulated.

A representative light micrograph of a Goldner's trichrome-stained section of the caries-affected dentin/adhesive interface is presented in Figure 3. No orange stain was observed at the caries-affected dentin interface, indicating the absence of complete infiltration anywhere throughout the interface. The red zone (~8 to 18 μm wide throughout Fig 3) represents the zone where adhesive was able to partially infiltrate but not fully encapsulate the collagen, leaving it exposed to react with Goldner's stain.

Based on the qualitative assessment, adhesive infiltration was superior for the noncarious dentin specimen as indicated by the presence of the zones with orange stain, which represent areas of complete collagen encapsulation.

Discussion

Principles of conservative dentistry advocate the removal of the outer caries-infected dentin

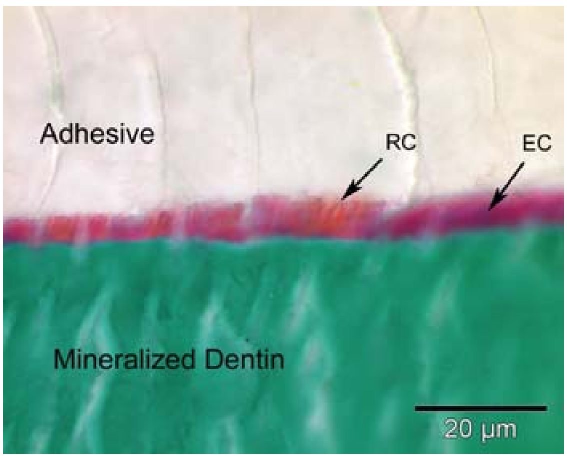

Figure 2. Photomicrograph (100× magnification) of the noncarious dentin/adhesive interface stained with Goldner's trichrome: mineralized dentin (green); adhesive (beige); collagen with resin adhesive infiltration (orange) indicated at RC; and exposed collagen (red) indicated at EC.

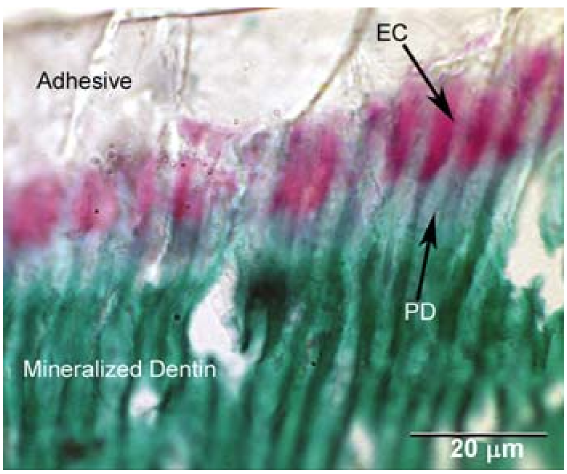

Figure 3. Photomicrograph (100× magnification) of the caries-affected dentin/adhesive interface stained with Goldner's trichrome: mineralized dentin (green); adhesive (beige); partially demineralized dentin (pale green) indicated at PD; and exposed collagen, EC.

while conserving the remineralizable inner caries-affected dentin. Adhesive bonding to caries-affected dentin has primarily been investigated by means of bond strength studies. Yoshiyama reported a 40% drop in bond strength to caries-affected dentin when compared with noncarious dentin.[21] Ceballos et al reported lower bond strength to caries-affected dentin when both self-etch and total-etch adhesive systems were used.[22] Nakajima et al also reported that bond strength of the same adhesive to caries-affected dentin was always less than that to noncarious dentin regardless of the strength of the etchant.[23] While such investigations allow comparisons between products and the effect of caries-affected dentin on in vitro bond strength, they do not provide insight into what potential differences in the caries-affected dentin/adhesive interface might be associated with decreased bond strength.

If composite resins are to be considered a viable core buildup material, the quality of the bond formed at the caries-affected dentin/adhesive interface must be addressed. Under in vivo conditions this bond has to withstand occlusal forces and polymerization shrinkage. In addition, it can be the first defense against substances that may penetrate and ultimately undermine the buildup restoration. The durability of the dentin/adhesive bond is directly related to the quality of the hybrid layer that connects the bulk adhesive to the subjacent, intact dentin.

The results from the current investigation suggest that for noncarious dentin, and under conditions simulating those of the oral cavity, the dentin/adhesive interface consisted of regions of more complete collagen encapsulation as indicated by the orange stain, surrounded by regions of exposed collagen with less-than-adequate resin infiltration (exposed collagen zone mean width $\sim 6\ \mu m$). Furthermore, within each specimen, the thickness of noncarious dentin/adhesive interface appeared fairly consistent.

In contrast, the results from the caries-affected dentin specimen suggest that the dentin/adhesive interface consisted of regions of exposed collagen (red stain) with less-than-adequate adhesive infiltration; no orange stain was observed indicating no regions with complete collagen encapsulation. Again, as compared with the noncarious dentin/adhesive interface specimen, there was greater variability in the thickness of the caries-affected/dentin interface within each specimen.

The wider zone of exposed collagen in the caries-affected dentin specimen may be related to a number of factors. Caries-affected dentin is known to be partially demineralized and more susceptible to acid etching. For example, because the caries-affected dentin is partially demineralized, there is less mineral to be removed with the acid etchant and thus, a deeper demineralized zone is produced.[24] Furthermore, Fusayama reported that the caries-affected dentin is not sclerotic but is a hypomineralized, softened, and more porous dentin.[24] This more porous substrate may allow deeper penetration of the acid etchant, resulting in a deeper demineralized zone.

With a deeper zone of demineralization, it is more difficult for the adhesive to completely penetrate the exposed collagen matrix. In addition, penetration of the adhesive into the demineralized caries-affected dentin could be further complicated by changes in the collagen associated with the caries process. Exposure of the dentin collagen matrix to caries-related acids results in denaturation of the collagen and a decrease in cross-linkage.[25] It is suggested that further exposure of the collagen to acid etching may lead to collagen phase transition into a gelatinous matrix. The formation of this gelatinous matrix may be associated with the previously reported decrease in the permeability of caries-affected dentin.[17]

Evidence of inadequate adhesive penetration into the demineralized dentin may be related to the in vitro decrease in bond strength associated with caries-affected dentin.[21-23] The results of this investigation would also suggest that under in vivo conditions such inadequate adhesive penetration could also lead to bond degradation and subsequent recurrent caries or restoration debonding. This naked collagen may be penetrated and degraded by various exogenous substances, including bacterial proteases, thereby threatening the longevity of the bond.[26-28]

In addition, the greater variability in the width of the caries-affected/dentin interface is potentially related to the removal of uneven amounts of the caries-affected dentin during caries excavation. During caries-affected dentin removal, the investigator used clinical judgment and tactile sensation to limit the excavation to the softer carious dentin. Since caries removal is not a precise procedure, varying amounts of caries-affected dentin might be left. Clinically, this presents another challenge; some clinicians may be bonding to mineralized dentin, while others to highly demineralized dentin. This variability could very well contribute to inconsistent and unpredictable adhesion quality.

Interestingly, a zone of partially demineralized dentin (pale green zone) was noted below the exposed collagen zone (~4 μm) of the caries-affected dentin specimens. This zone could simply be due to the residual effect of caries solution. As the acidic caries solution penetrated deeper into dentin, its concentration decreased, resulting in a demineralization gradient: demineralization that decreases from the surface of the caries-affected dentin into the deeper unaffected dentin. Although a zone of partially demineralized dentin was also noted in the noncarious specimen, it was much narrower (~1 μm). While this narrower zone in noncarious dentin is most likely the result of the residual effect of the acid etchant, the wider zone of partially demineralized dentin subjacent to the exposed collagen in the caries-affected specimen is probably due to a combination of residual effects from the acidic caries solution and the acid etchant.

The presence of multiple zones (encapsulated collagen, exposed collagen, and partially demineralized dentin) associated with the interface raises a concern over the integrity of the bond. The presence of different zones implies a wider variability in the elastic modulus throughout the interfacial structures. Katz et al used scanning acoustic microscopy to examine the micromechanical properties of the dentin/adhesive interface.[29] They reported the elastic moduli of the components of the interface as follows: partially demineralized dentin, 13 GPa; unprotected protein, <2 GPa; compared with mineralized dentin, 28 GPa. The variability of the elastic modulus within the dentin/adhesive interface raises the question of whether this zone will be able to withstand concentrated stresses at the dentin/restorative material interface. It is theorized that the hybrid layer may act as a buffer to dissipate stresses formed as a result of polymerization shrinkage, occlusal loading, and thermal expansion and contraction;[30] however, the ability of a poor-quality dentin/adhesive interface to act as a stress absorber without breakdown is yet to be determined.

Even though this investigation has attempted to evaluate the quality of adhesive infiltration at the caries-affected dentin/adhesive interface under conditions simulating the oral cavity, as with any laboratory investigation, there are some associated limitations. Artificial caries was used rather than actual caries in order to reduce some of the variables associated with the caries process. In addition, although only one adhesive was used in the investigation, one of the goals of this initial caries-affected dentin/adhesive interface investigation was to refine methodologies that can be used for future studies.

Future caries-affected dentin/adhesive investigations should include teeth with actual caries. Additional aspects to be considered would be self-etching adhesive systems, which tend to have a higher pH than conventional etchants. The differences in the acidity of the self-etch systems may be reflected in improved adhesive penetration and less exposed collagen in the caries-affected specimen. Another project could also examine whether chemomechanical caries removal with products such as Carisolv®[31] would more consistently remove the caries-affected dentin and thus result in a better dentin/adhesive interface.

The results of this investigation clearly demonstrate the complexity of the caries-affected dentin/adhesive interface. This research has highlighted some of the clinical implications and challenges facing dentists when restoring excessively carious teeth with bonded core-build materials. As indicated by the results of this study and the previous reports of weaker tensile bond strengths,[21-23] there appears to be less-than-ideal adhesion to caries-affected dentin and perhaps dentists should not depend solely on adhesives for retaining the composite core buildup restoration. The addition of mechanical retention such as grooves, potholes, or retaining pins should be considered a viable option to ensure adequate retention. In addition, placement of the crown margin beyond the core buildup/tooth interface should be emphasized and may reduce degradation of the bond between the core buildup and the remaining tooth structure.

It is evident that multiple factors play a role in the quality of the dentin/adhesive interface, including the adhesive system, the substrate, the clinician, and the surrounding environment. Good knowledge of such factors and careful case selection for bonded restorations, including core buildups, are critical to the longevity and success of the bonded restoration. The adhesive bonding materials are far more technique-sensitive than manufacturers have led clinicians to believe.

Conclusions

The results suggest that the caries-affected dentin/adhesive interface was of inconsistent thickness and that there was an associated wider zone of exposed collagen. In contrast, the thickness of the dentin/adhesive interface associated with noncarious dentin was of a more consistent thickness with a narrower zone of exposed collagen. It is clearly evident that the characteristics of the dentin substrate have a substantial effect on the structure of the dentin/adhesive interface.

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