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Resin Permeation into Acid-conditioned, Moist, and Dry Dentin: A Paradigm using Water-free Adhesive Primers

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Abstract. Preservation of the morphological integrity of demineralized dentin collagen in its hydrated state may account for the success observed in wet-bonding procedures. This study investigated the micromorphological differences between moist- and dry-bonding techniques with the use of: (a) Aelitebond, an alcohol-based, water-free, single-component dentin adhesive primer system; and (b) a water-free, acetone-based experimental primer similar to the acetone-based, water-containing All-Bond 2, a two-component primer system. In the wet groups, acid-conditioned dentin surfaces were blotted so that they remained visibly moist prior to bonding. In the dry groups, dentin surfaces were air-dried for 30 sec. Following the bonding procedures, dentin discs in each group were laminated together by means of a chemical-cure resin and processed for scanning electron microscope (SEM) and transmission electron microscope (TEM) examination. Conditioning with 10% H₃PO₄ for 20 sec. produced complete demineralization of the outer dentin. In the wet groups, banded collagen and interfibrillar spaces could be observed at the surface of the acid-conditioned dentin. Complete wetting of the loosely arranged collagen fibrils by the resin resulted in the formation of a hybrid layer. In the dry groups, only a very thin hybrid layer was observed on the dentin surface, along the walls of the tubules, and along the course of their lateral branches. The absence of banded collagen and interfibrillar spaces within these areas suggested the existence of a collapsed dentin matrix along various liquid-vapor boundaries that restricted resin permeation into the subsurface intertubular matrix, producing an incompletely infiltrated "hybridoid region".

Key words: acid-conditioned dentin, moist and dry bonding, resin permeation, hybridoid region.

Introduction

Many of the current generation of dentin adhesives depend on the permeation of hydrophilic resin into chemically conditioned dentin (Erickson, 1992; Van Meerbeek *et al.*, 1992; Eick *et al.*, 1993). The application of acid conditioners removed the smear layer (Lee *et al.*, 1973; Bowen, 1978; Berry *et al.*, 1987) and depleted the outermost region of dentin of its inorganic phase (Chiba *et al.*, 1989; Inokoshi *et al.*, 1990; Ruse and Smith, 1991; Ikami *et al.*, 1993; Tam and Pillar, 1994). Diffusion of resin into the demineralized, collagen-rich fibrous network resulted in "molecular entanglement of dental components" (Nakabayashi *et al.*, 1995), producing a hybrid layer of resin-reinforced dentin (Nakabayashi, 1982). The existence of the hybrid layer *in vivo* has since been consistently demonstrated (Nakabayashi *et al.*, 1992a, 1995; Gwinnett and Kanca, 1992a; Tay *et al.*, 1994, 1995a; Walshaw and McComb, 1994).

Maintenance of the structural integrity of the collagen fibrils and micro-channels within the demineralized network facilitated optimal permeation of adhesive monomers (Nakabayashi *et al.*, 1982). The presence of either denatured smear layer collagen remnants or denatured dentin surface collagen following acid application had been equated with a superficial, electron-dense zone within the hybrid layer that hindered resin permeation through the rest of the demineralized intertubular matrix (Van Meerbeek *et al.*, 1993). Inclusion of ferric chloride in a citric acid conditioner (Mizunuma, 1986) or a salicyclic acid derivative in the primer (Hosoda *et al.*, 1989) has been used as a chemical stabilizer of exposed collagen fibrils. Also along the line of chemical stabilization was the use of 2-hydroxyethyl methacrylate (HEMA) to reverse or re-expand a collapsed, demineralized dentin surface (Sugizaki, 1991), by improving the permeability of the bonding substrate (Nakabayashi and Takarada, 1992; Nakabayashi *et al.*, 1992b; Watanabe *et al.*, 1993; Mizuno *et al.*, 1994).

Recently, improvement in bond strength was observed when some hydrophilic monomers were applied to moist dentin (Gwinnett, 1992; Kanca, 1992; Perdigao and Swift, 1994). The presence of moisture appeared to play a crucial

role in preventing the demineralized surface collagen from collapsing following dehydration and accompanying surface tension stress (Gwinnett, 1994a). SEM studies of critical-point-dried specimens demonstrated that the physical integrity of the demineralized collagen could be preserved following the application of mild acids to dentin (Pashley *et al.*, 1993; Titley *et al.*, 1994). By means of an "environmental" SEM and cinematographic examination, it was found that the fibrous intertubular collagen network that initially accompanied moist, acid-conditioned dentin collapsed abruptly to form a smooth, featureless layer when the last trace of water evaporated from the dentin surface (Gwinnett, 1994b). However, remoistening dentin following a period of desiccation challenge of up to 30 seconds did not compromise the bond strength of an acetone-based adhesive to acid-conditioned dentin (Gwinnett, 1994a). To date, structural information on bonding to moist and dry dentin was all derived from SEM studies, and comparative ultrastructural data were lacking.

The purpose of this investigation was to compare the micromorphologic features of the resin-dentin interface when hydrophilic resin dissolved in acetone and/or ethanol was applied to acid-conditioned dentin under moist- and dry-bonding conditions. This was accomplished through an interactive study using SEM and TEM. To avoid the potential complication of creating a "pseudo-dry" condition resulting from re-wetting of briefly air-dried dentin (Gwinnett, 1994a) by the presence of water in some commercially available adhesive systems, we used a water-free, alcohol-based system as well as a water-free, experimental version of a commercially available acetone-based system as models.

Materials and methods

Twenty-four erupted, non-carious, human third molars, stored in 0.5% chloramine T, were used within one month following extraction. The roots were severed along the cemento-enamel junction by means of a low-speed machine equipped with a rotating diamond-impregnated copper disc under copious water supply, followed by the removal of occlusal enamel by means of a second parallel section. The bonding surface of the resulting dentin disc, from 1 to 1.5 mm thick, was then prepared with 600-grit silicon carbide sandpaper used to produce a flat surface devoid of enamel remnants or pulp exposures. Discs were randomly divided into four treatment groups, each containing six discs. The dentin discs were all conditioned with 10% phosphoric acid gel (All-etch semigel, Bisco, Itasca, IL) for 20 sec and rinsed with distilled water for 20 sec.

Aelitebond (Bisco, Itasca, IL; batch # 089417), an alcohol-based, water-free, single-component dentin adhesive primer system containing diarylsulfonatedimethacrylate (DSDM) was used in the first two groups.

DSDM moist group

After the acid conditioner was rinsed off, excessive water on each dentin disc was blotted (Kanca technique) with a small piece of tissue paper (Kimwipes, Kimberly Clark Corporation,

Roswell, GA). Two coats of primer were immediately applied followed by gentle blowing with oil- and dust-free air (Falcon Safety Products, Inc., Branchburg, NJ) to cause the alcohol in the primer to evaporate. Bonding resin was then brushed onto each primed dentin disc, followed by light-curing for 20 sec by means of a Max Lite (L.D. Caulk/Dentsply, Milford, DE) with the output intensity registered by a light meter (Cure Rite, L.D. Caulk/Dentsply, Milford, DE).

DSDM dry group

After the acid conditioner was rinsed off, each dentin disc was air-dried in a stream of oil- and dust-free air, blown horizontally across the dentin surface for 30 sec. The application of adhesive primer and bonding resin was exactly the same as for the moist group.

An experimental, acetone-based, dual-cure, two-bottle primer system, being a modified version of the commercially available All-Bond 2 (Bisco, Itasca, IL), was used for comparison. The primer A (2% sodium salt of N-p-tolyglycine-glycidyl methacrylate) used in this study was devoid of the 17.4% water present in the commercial product. Except for the modified version of primer A, the remaining components used throughout the experiment were from a conventional All-Bond 2 kit (Batch # 029154).

Experimental formulation moist group

After the acid conditioner was rinsed off, excessive water on the dentin surface was blotted with a small piece of tissue paper. Equal drops of experimental primers A and B (14% biphenyl dimethacrylate) were dispensed and mixed for 5 sec. Multiple coats (8 to 10) were applied to each dentin disc until the primer in the well was used up. Each disc was gently air-dried with oil- and dust-free air for 20 sec so that the acetone and ethanol would evaporate completely. The primers were light-cured for 20 sec, at which time a shiny surface was noted. A thin layer of bonding resin was then applied and polymerized for an additional 20 sec.

Experimental formulation dry group

After the acid conditioner was rinsed off, each dentin disc was air-dried for 30 sec in the manner already described. The priming and bonding procedure was exactly the same as in the moist group.

The six treated dentin discs from each group were bonded together into 3 disc-pairs by means of a thin layer of chemical-cure bonding resin, similar to the sandwich technique described by Inokoshi *et al.* (1990). The bonded disc-pairs were sectioned perpendicular to the flat dentin surface into approximately equal halves by means of a diamond-impregnated copper disc under copious water supply. One half of each disc-pair was used for SEM and the other half for TEM examination.

Two strips, approximately 1.5 mm wide, from one half of each disc-pair were prepared for SEM. Each strip was attached to the stub of a specimen grinder (Model 623, Gatan Inc., Pleasanton, CA), and the bonded interface was polished under running water with silicon-carbide papers of, successively, 600, 1000, and 1200 grit, followed by 1- μ m-size polishing sheets (Imperial Polishing Film, 3M Microfinishing Systems, St. Paul, MN). One mounted polished strip from each of the 3 disc-pairs

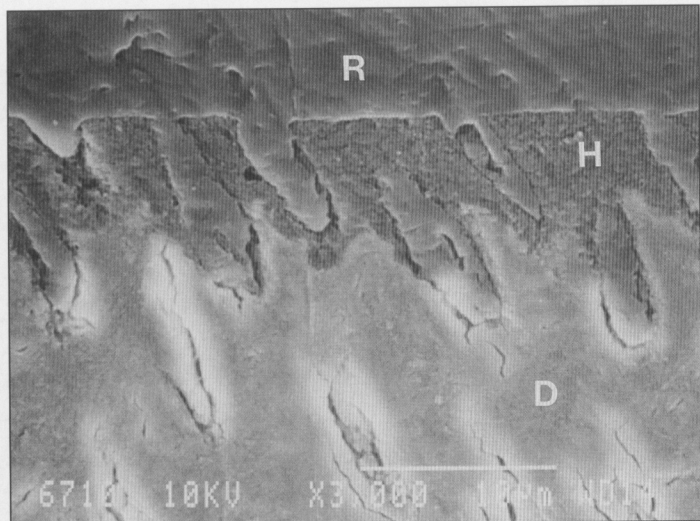


Figure 1a. Scanning electron micrograph of the resin-dentin interface in the DSDM moist group following plasma-etching. A hybrid layer (H) of about 5 μm was observed. Uneven removal of material along the interface, with the softer hybrid layer removed to a greater extent, resulted in a step between the resin layer (R) above and the intertubular dentin (D) below. Bar = 10 μm .

was plasma-etched with microwave-activated argon at 208 kPa for 3 min under vacuum and water cooling (Plasma-Preen II, Model 862, Plasmatic Systems, Inc., North Brunswick, NJ), following a method reported by Hunter *et al.* (1993). Specimens were then ultrasonically cleaned in distilled water for 3 min.

The other polished strip from each of the 3 disc-pairs in the same group was rinsed in 0.01 N HCl for 20 sec. to bring the resin-dentin interface into relief through partial dissolution of the dentin. The prepared specimens were sputter-coated with gold in an ion sputtering machine (Fine coat, FC-1100, JEOL, Japan) at 1.2 kV and 5 mA for 8 minutes. Observations were performed in a scanning electron microscope (JXA-840, JEOL, Japan) operated at 10 to 15 kV.

The other half of each disc-pair was completely demineralized in an aqueous solution of ethylene diamine tetraacetic acid (EDTA) buffered with sodium hydroxide to a pH of 7, fixed in Karnovsky fixative for 4 hr, and rinsed with sodium cacodylate buffer. *En bloc* staining and post-fixation were performed simultaneously in a 1:1:1 by volume solution of: (a) 1% osmium tetroxide, (b) 0.1% ruthenium red, and (c) 0.2 M sodium cacodylate buffer, pH 7.3, at room temperature for 12 hr. Ruthenium red or its derivative as a negative stain has been shown to enhance the contrast of collagen (Luft, 1971; Garofalo *et al.*, 1993). Specimens were further rinsed three times in cacodylate buffer, dehydrated in an ascending ethanol series (from 30% to 100%), immersed in propylene oxide, and embedded in epoxy resin (TAAB 812 resin, TAAB Laboratories, UK). A 2-mm longitudinal strip was sectioned from the resin block, from which three 2 x 2 mm blocks were trimmed and re-embedded in epoxy resin to ensure proper orientation of the resin-dentin interface. Following examination of the semithin sections, 1 mm x 1 mm ultrathin sections were prepared by means of a diamond knife and collected on 100-mesh copper grids (TAAB Laboratories, UK). The grids were double-stained with 2% uranyl acetate for 10 min, followed by Reynolds' lead

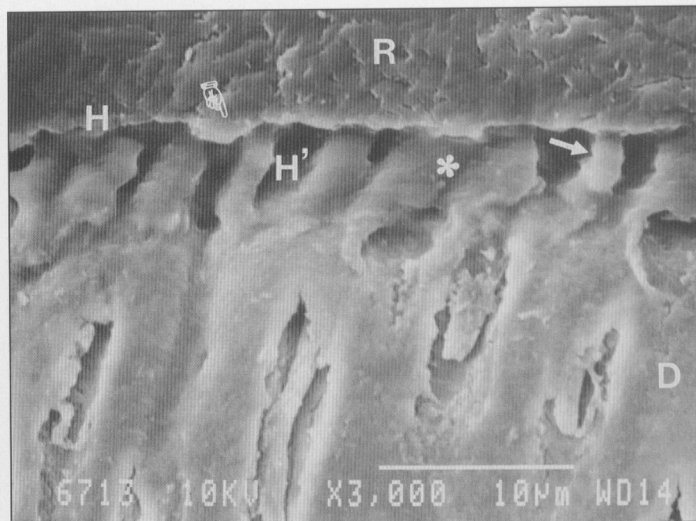


Figure 1b. Scanning electron micrograph of the resin-dentin interface in the DSDM dry group following plasma etching. A thin, surface hybrid layer (H) immediately beneath the resin layer (R) could be observed together with part of a broken resin tag (pointer). In the "hybridoid region" (H') below, differential removal of the softer, partially infiltrated collagen (asterisk) resulted in an incomplete layer, where exposed resin tags (arrow) could be seen entering the underlying dentin (D). Bar = 10 μm .

citrate for an additional 5 min. After being dried, they were examined with a transmission electron microscope (JEM-100, JEOL, Japan) operating at 80 kV.

Results

In the DSDM moist group, a hybrid layer of about 5 μm could be observed in the SEM following plasma etching, with resin tags traversing from the resin layer above into the undemineralized dentin below (Fig. 1a). In the dry group, only a very thin hybrid layer was observed beneath the resin layer, with the underlying zone consisting of voids as well as individual resin tags (Fig. 1b). This incompletely infiltrated zone will be referred to as the "hybridoid region", to differentiate it from the thin, superficial hybrid layer. Material appeared to have been selectively removed, leaving behind resin tags as well as remnants of an amorphous material between the resin tags. The resin-dentin interface in the moist and dry groups, disclosed with acid dissolution, showed some features similar to those described above.

In the experimental formulation moist group, a hybrid layer of approximately 5 μm , brought into relief after acid dissolution, confirmed the SEM results disclosed by plasma etching (Fig. 2a). In the dry group, three different zones could be observed (Fig. 2b). A thin surface and basal hybrid layer was present, with a texture similar to that observed in the moist group. Lateral branches of the dentinal tubules were closely associated with the basal hybrid layer. The middle "hybridoid region" contained voids and incompletely infiltrated collagen that appeared collapsed and shrunken following air-drying of the specimens.

A TEM view of the resin-dentin interface in the DSDM moist group showed a hybrid layer that was clearly

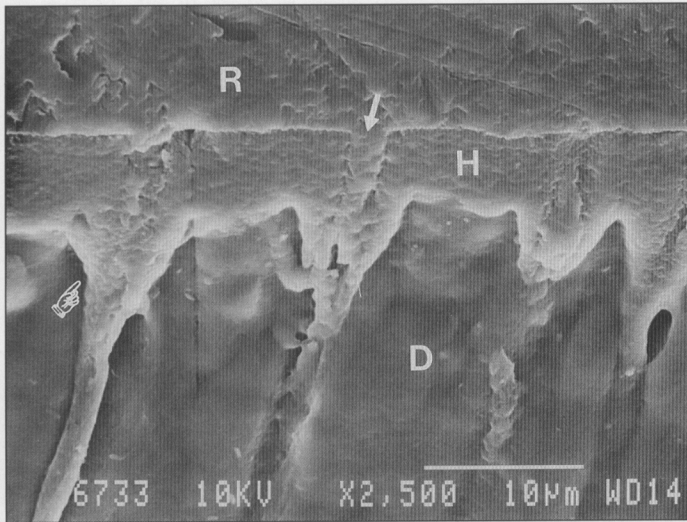


Figure 2a. Scanning electron image of the resin-dentin interface in the experimental formulation moist group that has been acid-rinsed. A distinct hybrid layer (H) of about 5 µm was observed. The resin layer (R) above could be seen extending as a resin tag (arrow) through the hybrid layer. A wedge-like extension of the hybrid layer formed a circumferential cuff around the periphery of the resin tag (pointer). The underlying dentin (D), being partially demineralized by acid-rinsing in HCl for SEM visualization, collapsed on air-drying and appeared at a lower level, revealing the resin tags in relief. Bar = 10 µm.

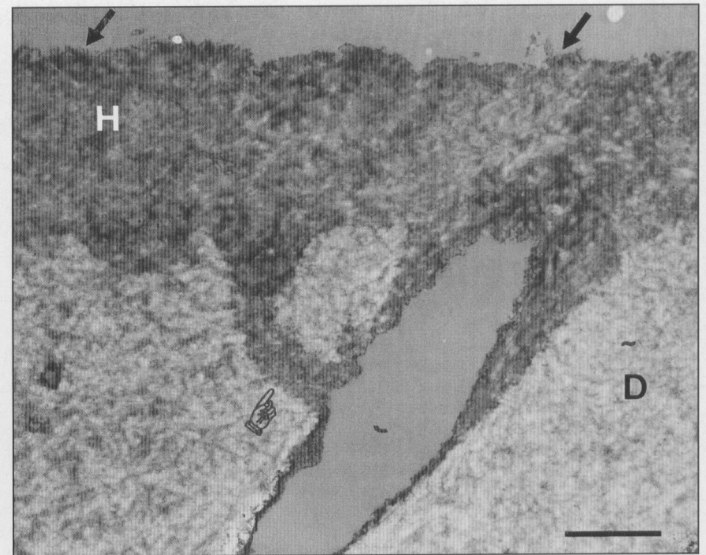


Figure 3a. Transmission electron micrograph of an overview of the resin-dentin interface in the DSDM moist group. Tiny protusions from the hybrid layer (H) resulted in an uneven and rugged border (arrows). The basal portion of the hybrid layer (arrowheads), well demarcated from the underlying dental matrix (D) that was demineralized in EDTA for TEM preparation, was devoid of an electron-dense zone that was suggestive of the presence of encapsulated minerals. Pointer: contribution of resin infiltration through anastomosis of a lateral branch. Bar = 2 µm.

distinguishable from the underlying dentin (Fig. 3a) and consistent in its appearance from the surface to the base. Although a sharp demarcation existed between the base of

the hybrid layer and the underlying EDTA-demineralized dental matrix, there was no evidence of a partially demineralized zone of dentin at the base of the hybrid layer.

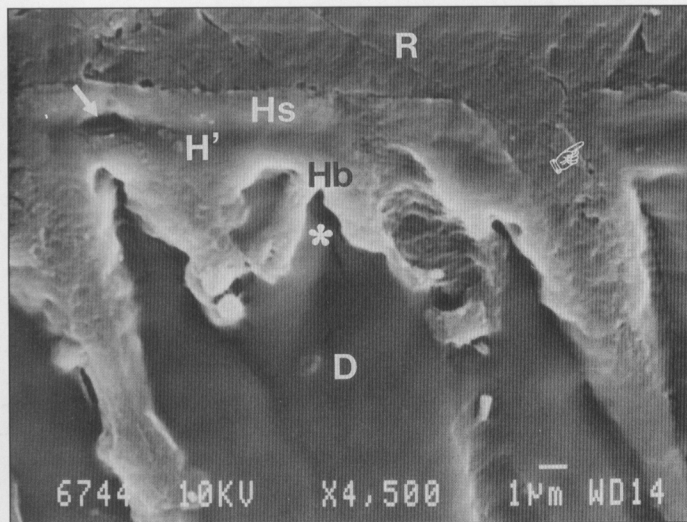


Figure 2b. Scanning electron micrograph of the resin-dentin interface in the experimental formulation dry group after acid-rinsing, in which three zones could be distinguished: a surface (Hs) and a basal (Hb) hybrid layer sandwiching a middle, collapsed "hybridoid region" (H'). The surface hybrid layer represented the result of limited vertical intertubular monomer permeation through the acid-conditioned, surface intertubular dentin. The basal hybrid layer represented the contribution from dental tubule anastomosis (asterisk). The surface and basal hybrid layers were connected along the periphery of a dental tubule (pointer) through circumferential intertubular permeation from the tubule lumen. Arrow, void within "hybridoid region"; R, resin layer; D, dentin matrix demineralized by acid-rinse in 0.01 N HCl. Bar = 1 µm.

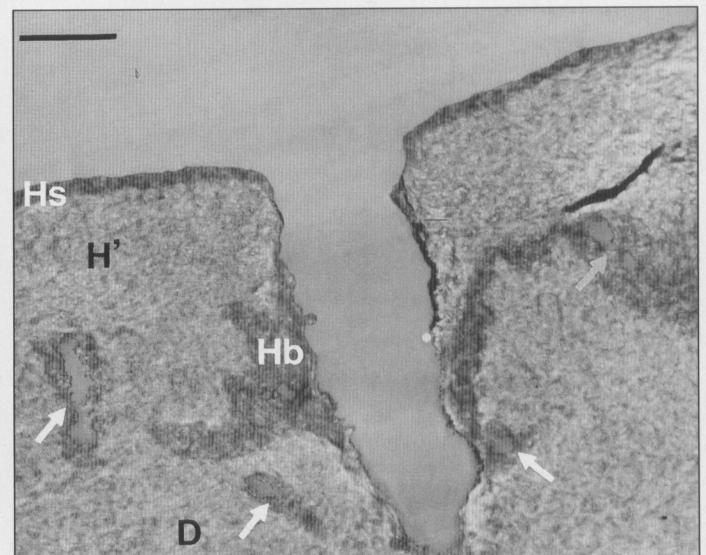


Figure 3b. Transmission electron micrograph of an overview of the resin-dentin interface in the DSDM dry group. A thin, electron-dense zone was observed along the dentin surface (Hs). The basal electron-dense zone (Hb) was discontinuous and could be found circumferentially around the lateral branch of a dental tubule (arrows). In areas where discontinuity of the basal electron-dense zone occurred, there was no sharp demarcation between the less-electron-dense "hybridoid region" (H') and the underlying dental matrix (D) that was demineralized in EDTA to facilitate TEM preparation. Bar = 2 µm.

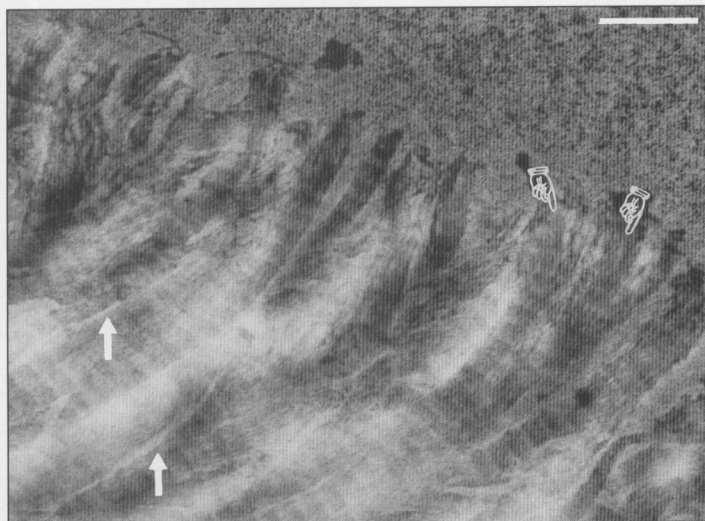


Figure 4a. Transmission electron micrograph of the surface of the hybrid layer in the DSDM moist group. Collagen fibrils could be traced to the surface of the hybrid layer, where they were arranged at right angles to the surface. Unraveling of microfibrils from the severed ends of some collagen fibrils could be observed (pointers). Interfibrillar channels (arrows) between adjacent collagen fibrils were evident. Bar = 250 nm.

Air-drying the dentin surface for 30 sec prior to the application of the dentin adhesive resulted in an incompletely infiltrated layer, wherein a "sandwich arrangement" could be distinguished, consisting of three zones (Fig. 3b). A thin electron-dense hybrid layer, similar to the staining characteristics of that in the moist group, was evident along the surface and basal portions, with a less-electron-dense "hybridoid region" sandwiched in between. The basal hybrid layer, being roughly parallel to the surface hybrid layer, was discontinuous and with a tendency to form around the lateral branches of the dentinal tubules, which were filled with resin.

At higher magnification, the surface of the hybrid layer in the DSDM moist group consisted of banded collagen fibrils that could be traced at right angles to the dentin surface (Fig. 4a). Unraveling of individual microfibrils at the severed junction resulted in the appearance of loose strands, with the cross-striations from longitudinally sectioned collagen fibers clearly visible immediately underneath. Interfibrillar channels between adjacent collagen fibrils were evident. A view from a similar location in the DSDM dry group showed a surface zone where diffuse electron-dense patches were scattered within an area devoid of both collagen banding or interfibrillar spaces (Fig. 4b). Further down within the "hybridoid region", banding within collagen fibrils as well as interfibrillar spaces was evident. Similar features were observed along the basal portion as well as around the lateral branches (Fig. 4c).

An overall TEM view of a longitudinal section through a dentinal tubule in the experimental formulation moist group is shown in Fig. 5a. Within the hybrid layer, features similar to those observed in the DSDM moist group were present. In addition, remnants of the lamina limitans, seen as an electron-dense sheath, appeared within the resin tags. In a

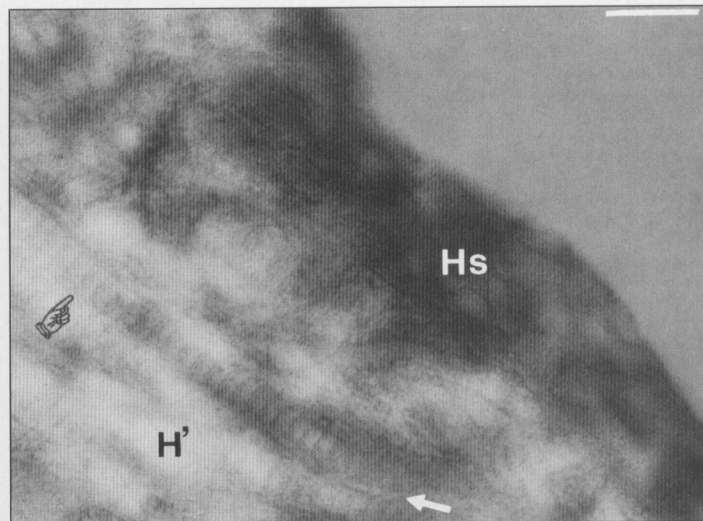


Figure 4b. A transmission electron micrograph of the dentin surface in the DSDM dry group. Collagen banding (pointer) or interfibrillar spaces (arrow), non-existent at the surface electron-dense zone (Hs), became more prominent within the sub-surface "hybridoid region" (H'). Bar = 250 nm.

comparable overall view of the experimental formulation dry group, there was a gradient of electron-density extending from the dentin surface into the subsurface "hybridoid region" (Fig. 5b). Electron-dense zones were found around the periphery of the dentinal tubules as well as their lateral branches, which were filled with resin.

In the moist groups, resin occupied initially the entire lumen at the orifice of the dentinal tubule. Further down, these short solid resin tags were replaced by resin globules, being attached to the periphery of the dentinal tubules *via* a thin resin sheath (Fig. 6a). In contrast, long solid resin tags could be observed in the dry groups (Fig. 6b). At higher magnification, diffusion of resin through the very thin, electron-dense lamina limitans resulted in the entrapment of a layer of amorphous material that appeared more like denatured protein remnants than partially dissolved hydroxyapatite crystallites (Fig. 6c).

Discussion

The dental literature is rich with scientific articles describing the interface between restorative materials and tooth tissue since Kramer and McLean published the first paper in 1952. Recent studies have added valuable information on the topographical or ultrastructural features of the resin-dentin interface, with the use of adhesive systems that compared different treatment philosophies of the smear layer and smear plugs (Nakabayashi, 1982, 1992; Abe, 1986; Takarada, 1990; Eick *et al.*, 1991, 1992, 1993; Erickson, 1992; Van Meerbeek *et al.*, 1992, 1993; Inokoshi *et al.*, 1993; Watanabe *et al.*, 1994). Reports on the structural aspects of the reaction of adhesive systems to moist- and dry-bonding conditions following the removal of the smear layer and the smear plugs with acid conditioners had been relatively scarce (Gwinnett and Kanca, 1992b; Titley *et al.*, 1994).

In the present study, microstructural features of the water-

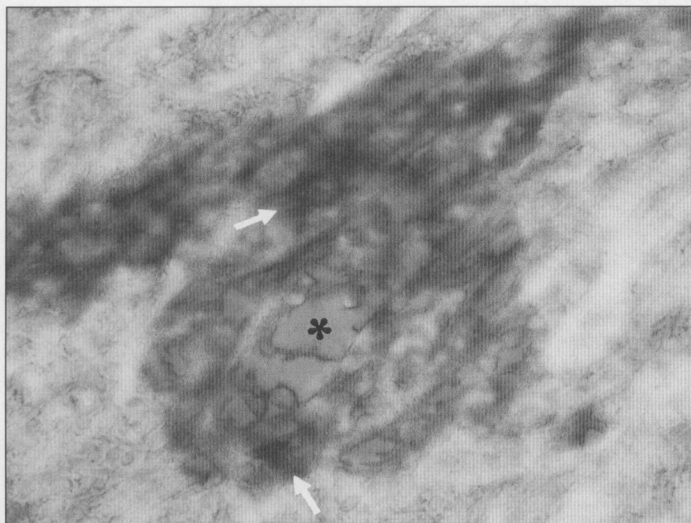


Figure 4c. A transmission electron micrograph of the basal electron-dense zone in the DSDM dry group. A layer of electron-dense, resin-infiltrated, partially denatured collagen (arrows) could be seen around the lateral branch of a dentinal tubule (asterisk). Bar = 500 nm.

free resin systems were strikingly similar in their respective moist and dry groups. In contrast to the moist groups, the dry groups showed evidence of incomplete resin infiltration, with

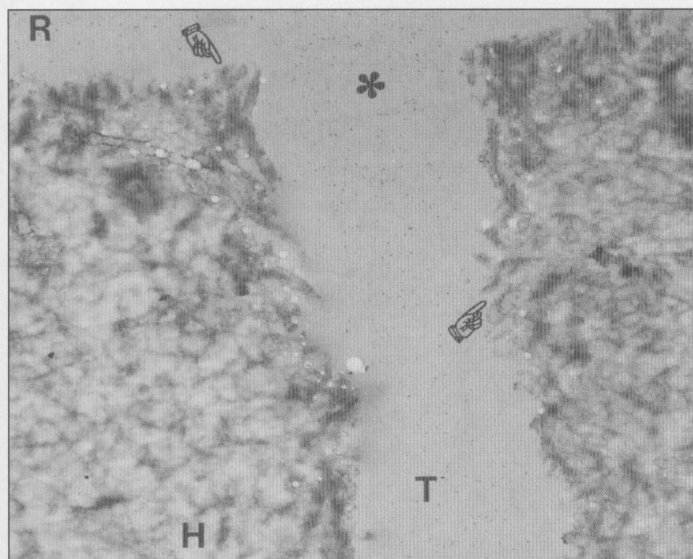


Figure 5a. Transmission electron micrograph of an overview of the resin-dentin interface from the experimental formulation moist group. The surface of the hybrid layer was devoid of any remnants of the smear layer, with the patency of the tubular orifice well-maintained (asterisk). Collagen could be seen protruding from the surface of the intertubular dentin as well as from the walls of the tubular lumen (pointers). There appeared to be a uniform penetration of the adhesive resin throughout the hybrid layer (H), with the absence of an electron-dense basal zone along the demineralization front (arrowheads). Remnants of the lamina limitans (arrows), the limiting membrane of the tubular lumen, could be seen partially detached from its peripheral location, resulting in a folded appearance further down the resin tag. R, resin layer; T, resin tag; D, underlying dentinal matrix that has been demineralized in EDTA for TEM preparation. Bar = 2 μ m.

the exposed, uninfiltated collagen eroded to a greater extent during argon ion-etching (Miller *et al.*, 1994), contributing to a so-called "hybridoid region". Using sodium hypochlorite to remove incompletely infiltrated collagen within the layer of resin impregnated dentin, researchers have previously reported a similar zone with a dry-bonding technique (Wang and Nakabayashi, 1991), or with a wet-bonding technique when dentin surfaces were conditioned with 65% phosphoric acid (Kato *et al.*, 1994). Dentin conditioning by means of phosphoric acid with a concentration beyond 35% resulted in denaturation of the demineralized collagen (Mizunuma, 1986; Ikami *et al.*, 1993; Reeve *et al.*, 1994). Restricted resin permeation in the presence of denatured collagen (Pashley *et al.*, 1993) could explain why only a very thin, surface hybrid layer was observed previously in a wet-bonding technique (Kato *et al.*, 1994).

It was not clear from the SEM results why an additional basal zone could be observed below the "hybridoid region" in the dry groups. TEM results for the dry groups, however, indicated that the staining in the "hybridoid region" resembled that of the dentin matrix underneath that had been demineralized in EDTA for TEM preparation. The basal zone, on the other hand, had staining similar to that of the surface zone, and was often associated with resin-filled lateral branches extending from adjacent dentinal tubules. Thus, certain sites within and along the basal portion of the "hybridoid region" could be better infiltrated if they were

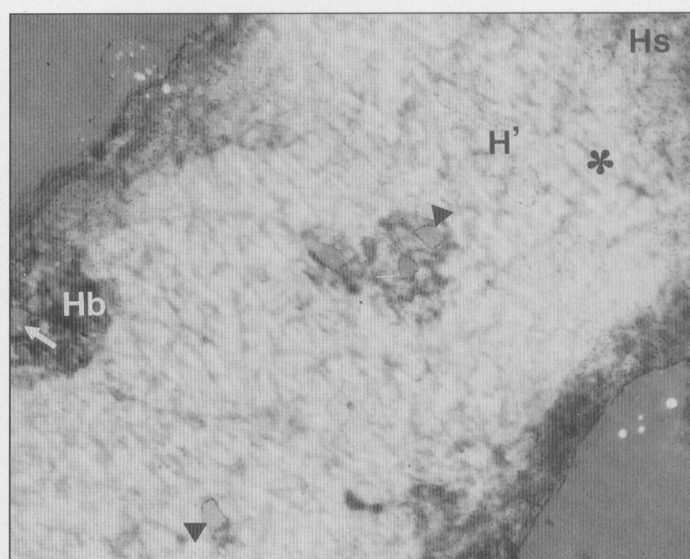


Figure 5b. Transmission electron micrograph from a specimen in the experimental dry group. The tubular orifice, being constricted (arrows) and obscured by some amorphous material (white asterisk), probably represented an artifact resulting from an oblique section through the curvature of a dentinal tubule. Within the resin tag (T), an electron-dense line representing the lamina limitans could be seen (pointers). The surface electron-dense zone (Hs) was continuous with similar zones along the periphery of the tubular lumen (K). Lateral branches in cross-sections (black arrowheads) and a longitudinal section, branching out from a dentinal tubule (white arrow), could be identified. The presence of an electron-dense zone around these lateral branches constituted the incomplete basal zone (Hb). The subsurface "hybridoid region" (H') could not be readily distinguished from the underlying EDTA demineralized dentin matrix (D). R, resin layer. Bar = 2 μ m.

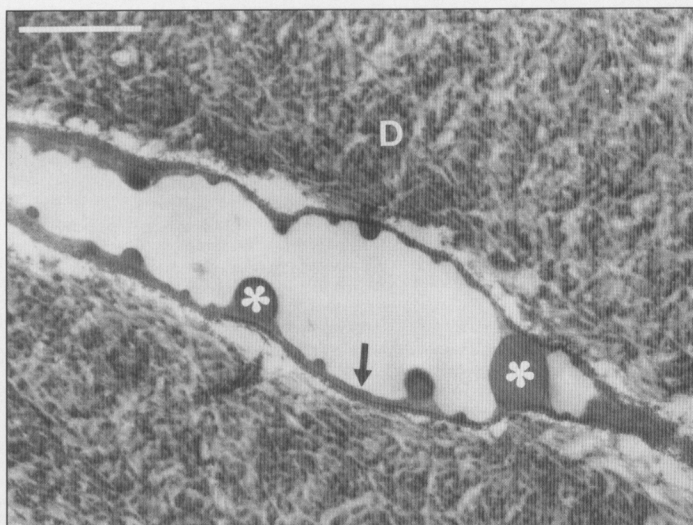


Figure 6a. Transmission electron micrograph of a dentinal tubule in the experimental formulation moist group, where resin globules (asterisks) were joined by a thin sheath of resin (arrow) to form a hollow resin tag. D, intertubular dentinal matrix resulting from EDTA demineralization for TEM processing. Bar = 1 μ m.

traversed by lateral branches from the dentinal tubules.

Pashley *et al.* (1993) distinguished between transdentinal permeation, *i.e.*, the intratubular penetration of resin to form resin tags, and intradentinal permeation, *i.e.*, the infiltration of resin into demineralized *intertubular* dentin. Dentinal tubule anastomosis through an interlocking network of resin-filled lateral branches has also been suggested as a potential contributing factor in micromechanical bonding to dentin (Chappell *et al.*, 1994). It is clear from the results of our study that intratubular and intertubular permeation was adequate under moist-bonding conditions, resulting in a continuous hybrid layer down to the demineralization front produced by the acid-dentin conditioners. Moreover, resin globules that were observed beyond short resin tags were similar to those previously reported in deep dentin *in vivo* (Tay *et al.*, 1994, 1995d). Although effective intratubular permeation in the dry groups resulted in the formation of longer, solid resin tags within the dentinal tubules, vertical intertubular permeation was incomplete, resulting in a thin surface hybrid layer limited superficially to 1 to 2 μ m. Circumferential intertubular permeation, being an extension of intratubular permeation through the periphery of the tubule lumina and their lateral branches, resulted in partial infiltration of the rest of the underlying demineralized dentin matrix. A thin hybrid layer was therefore observed along areas that were close to the course of the dentinal tubules and their lateral branches, leaving behind a subsurface, incompletely infiltrated "hybridoid region". Although remineralization of demineralized dentin collagen that retained its cross-banding (Kato and Fusayama, 1970; Miyauchi *et al.*, 1978; Tatsumi, 1989) has been reported to be possible, its occurrence is doubtful in these isolated regions within the circumpulpal dentin, in the presence of resin tags in the dentinal tubules and with the possibility of damage to the odontoblast processes that could have limited the access to calcium and phosphate ions and phosphophoryns (Butler

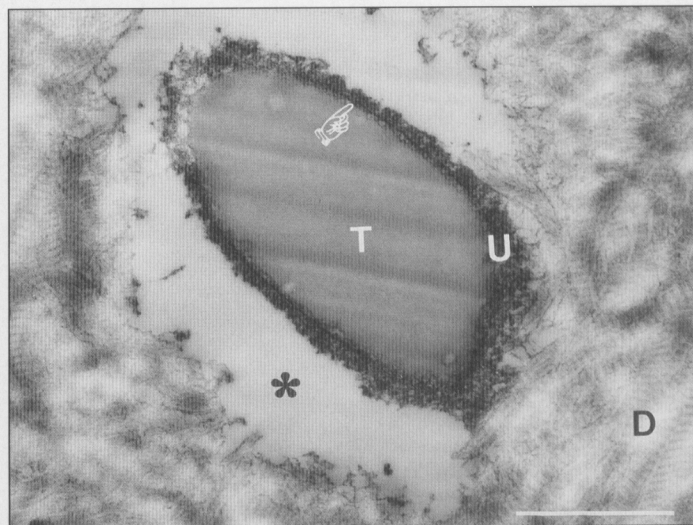


Figure 6b. Transmission electron micrograph of a solid resin tag (T) in the experimental formulation dry group. The resin has diffused through the lamina limitans (pointer), trapping a layer of electron-dense material (U) within the peritubular space (asterisk). D, intertubular dentinal matrix that was demineralized by EDTA. Bar = 1 μ m.

et al., 1992; Rabie and Veis, 1996). Furthermore, it is not known to what extent the resident noncollagenous proteins are extracted by different acids during the dentin-conditioning step (Pashley *et al.*, 1992), degraded in the absence of tissue turnover (Veis, 1993), or whether they are critical for the remineralization of demineralized dentin collagen (Takagi and Sasaki, 1986).

The bond between a restoration and tooth substrate might be equated with a chain, comprised of a series of links, which could only be as strong as its weakest link (Gwinnett, 1988). It is reasonable to assume that, in the dry groups, the weakest link within the resin-dentin interface resided within the hybrid layer (Kiyomura, 1987; Wang and Nakabayashi, 1991; Burrow *et al.*, 1993; Kato *et al.*, 1994). Pashley *et al.* (1992) and Sano *et al.* (1994) suggested that incomplete resin infiltration within the demineralized intertubular matrix resulted in a weak collagen-rich zone that is susceptible to hydrolysis and microleakage. On the other hand, properly infiltrated demineralized dentin (hybrid layer) has been shown to restore the ultimate tensile strength of mineralized dentin (Sano *et al.*, 1995), an event predicted by the cohesive plateau theory (Adamson, 1982), with values greater than those reported for adhesion of resin to dentin. It is likely that the adhesive bond in the wet groups would fail along the interface between the adhesive resin and the top of the hybrid layer, as has been observed in previous studies (Tagami *et al.*, 1993; Gwinnett and Yu, 1994; Tay *et al.*, 1995b).

Ultrastructural features observed in both dry groups were comparable with results obtained in the application of the Clearfil Liner Bond system to dry, acid-conditioned dentin (Van Meerbeek *et al.*, 1993), in which a similar electron-lucent zone could be observed between a surface and a basal electron-dense zone. In contrast, the hybrid layer observed in the moist groups in the present study was completely different, with no zonations and a notable

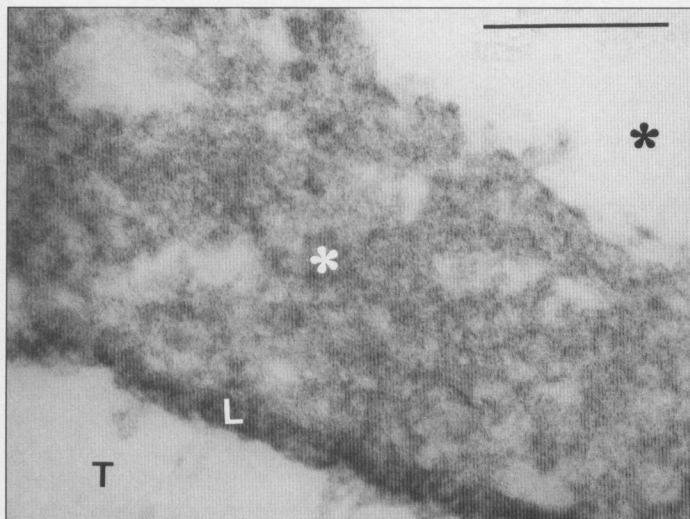


Figure 6c. Higher magnification of the electron-dense layer in Fig. 6b revealed the entrapment of a collection of amorphous material (white asterisk) by the resin following its diffusion through the lamina limitans (L) from the lumen of the dentinal tubule (T). Such a layer probably represented denatured proteins from the peritubular dentin. No resin-encapsulated hydroxyapatite crystallites could be observed. Black asterisk = peritubular space. Bar = 200 nm.

absence of a basal electron-dense zone attributed to partially demineralized dentin. There appeared to be complete demineralization with the 10% phosphoric acid, ending in a sharp demarcation between the zone of resin-infiltrated demineralized dentin and the underlying dentinal matrix which was subsequently demineralized and embedded in epoxy resin. This finding is in agreement with the work of Ikami *et al.* (1993). They also showed a thin layer of denatured collagen on the dentin surface but only after treatment with 38% phosphoric acid for 60 sec, an observation recently confirmed by Reeve *et al.* (1994). No denatured collagen was observed when dentin was conditioned with milder acids, an observation in common with the present study, where intact, banded collagen fibrils could be observed in moist groups right to the surface of the demineralized dentin.

If the dry group had been inspected without comparison with a moist group, it might be concluded that the electron-dense surface zone in the dry groups was either a result of denaturation of surface collagen by the acid conditioner or denatured collagen remnants from the original smear layer. It might also be concluded that the electron-dense basal zone consisted of resin-infiltrated, partially demineralized hydroxyapatite crystallites. While one cannot refute the possibility of the occurrence of a zone of partially demineralized dentin with the use of some conditioners (Nakabayashi *et al.*, 1992a, 1995; Van Meerbeek *et al.*, 1993), it was evident from the TEM that such a zone was absent in the present study. The presence of electron-dense zones along the dentin surface, at the periphery of the dentinal tubules, as well as around their lateral branches suggested a similarity among these regions. They were all located adjacent to the liquid-vapor interface through which water has to pass during the process of evaporation. Water

evaporating through and around the soft, demineralized collagen subjects it to crushing and tearing forces (Anderson, 1951) caused by the molecular linkage of water at the liquid-vapor boundary. The collagen fibrils protruding from the liquid surfaces could collapse and denature, resulting in a layer of amorphous material that restricted resin permeation (Pashley *et al.*, 1993).

Similar to the problem encountered by Van Meerbeek *et al.* (1993), visualization of resin penetration into the decalcified dentin was difficult to achieve by TEM alone. However, when the moist and dry groups were compared, resin infiltration was more complete in the moist groups, where loosely arranged collagen fibers probably provided little resistance to the infiltration of the resin dissolved in either acetone or ethanol. At the same time, the acetone or ethanol displaced the water within the interfibrillar channels into which the resin infiltrated and formed a close physical relationship with the more loosely arranged tissue microstructure. In the dry groups, the electron-dense layers probably represented an amorphous mass of denatured collagen that, in the absence of interfibrillar spaces, acted as a molecular sieve, leading to a localized increase in resin concentration within the surface zone, while at the same time restricting diffusion of resin through this zone into the subsurface "hybridoid region". Such a molecular sieve effect may be similar to that proposed for resin infiltration of enamel (Darling *et al.*, 1961).

It is speculated that water could be an essential component in some current adhesive systems, allowing desiccated collagen to be re-wetted prior to infiltration by bifunctional methacrylate components. Previous reports on the ability of HEMA to enhance the penetration capability of dentin substrates described the use of aqueous solutions of HEMA (Suzuki *et al.*, 1990; Nakabayashi and Takarada, 1992; Nakabayashi *et al.*, 1992b; Watanabe *et al.*, 1993; Mizuno *et al.*, 1994). Organic water-miscible solvents such as acetone, ethanol, and HEMA have been shown to exert a stiffening effect on demineralized dentin collagen (Pashley, 1996). Such chemical stabilization is useful when the collagen matrix is hydrated, allowing the integrity of the interfibrillar spaces to be maintained and facilitating resin infiltration. On the contrary, in the absence of water, chemical stiffening of a dehydrated and collapsed collagen matrix prevents its re-expansion and results in restricted resin infiltration. As illustrated in this study, application of "water-free primers" (*i.e.*, primers with no additional water included in the solvent) to air-dried dentin resulted in incomplete hybridization. It might be argued that desiccating the acid-conditioned dentin for 30 sec followed by the application of such "water-free primers" was far removed from the usual technique adopted in clinical practice, aggravating the results observed in the dry groups. However, by subjecting the specimens to such a degree of desiccation challenge, the authors wished to establish a paradigm for the behavior of these adhesives at one end of the spectrum—the overdry situation. There is probably a limit to the amount of moisture necessary for keeping the demineralized collagen stable so that the benefits of moist bonding can be optimized. Studies are currently being

conducted in our laboratories on the reaction of these adhesives to various degrees of surface wetness. Application of "water-containing primers" (i.e., hydrophilic resins dissolved primarily in acetone but with different amounts of water added by the manufacturers as part of the solvent) to an already-moist dentin surface, for example, represents the other end of the spectrum, resulting in an "overwet phenomenon" (Gwinnett *et al.*, 1996; Pang *et al.*, 1995; Tay *et al.*, 1995c; Wei *et al.*, 1995). In the presence of excessive water, some of these resins appear to undergo phase separation of the hydrophobic components of the resin composition, resulting in resin globule formation.

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