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What is This?

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Abstract. In an attempt to compare the morphology of the dentin adhesive interface and the wetting and penetration of the adhesive in relation to the dentin surface, we studied four dentin adhesive systems using scanning transmission electron microscopy (STEM) and energy-dispersive spectroscopy (EDS). 2-Hydroxyethylmethacrylate (HEMA), a monomer common to many commercial dentin adhesive systems, was altered to produce a thiolated analogue (HETMA). Sulfur, traceable by EDS and STEM, was substituted for the oxygen atom in the backbone of the HEMA molecule. The resulting analogue, with solubility parameters and other wetting and physical properties very similar to those of HEMA, was applied to four sets of tooth specimens, each pre-treated with a different primer or etchant. Three separate pre-treatments—nitric acid, maleic acid, and citric acid/ferric chloride—created a demineralized zone approximately 1 to 3 μm thick at the dentin surface. The HETMA was found to permeate freely into this zone when either of the latter two pre-treatments was used. However, the band of dentin that was demineralized by the nitric acid pre-treatment appeared impermeable to the HETMA. The fourth pre-treatment, an alcohol-based solution including the phosphorus acid ester PENTA and HEMA, modified the smear layer of the tooth slightly and did not appear to demineralize the dentin. HETMA applied to the specimens pre-treated with PENTA and HEMA was clearly in intimate contact with the dentin or modified smear layer; however, it did not penetrate or diffuse into these areas. It did flow into the dentinal tubules, as was also evident with each of the other systems. It was concluded that the acid pre-treatment of the dentin greatly influenced the wetting behavior of the dentin adhesive and thus could substantially affect the resultant bond strength of the dentin adhesive systems.

Key words: dentin bonding, STEM/EDS analysis, HEMA.

Introduction

There has been considerable research in recent years to develop a dentin adhesive that would produce adequate bond strength and be stable in the oral environment. It has been shown that a shear bond strength value of at least 17 MPa is necessary to compensate for the stress produced by the polymerization shrinkage of a resin composite (Davidson and DeGee, 1984). If intimate contact is obtained between the dentin adhesive and the dentin substrate, cohesive failure often occurs in the dentin when shear bond strengths of 17 MPa or greater are achieved during laboratory testing (Barkmeier and Cooley, 1991; Chappell et al., 1991; Eick et al., 1993a,b; Chappell and Eick, 1994). For clinically acceptable bond strengths to be obtained, it is generally agreed that the dentin adhesive must first wet the dentin surface (Erickson 1989, 1992; Van Meerbeek et al., 1992b; Eick et al., 1993a; Pashley et al., 1993) and then penetrate the demineralized layer produced during conditioner/primer treatment of the smear layer (Nakabayashi, 1982; Gwinnett and Kanca, 1992; Nakabayashi, 1992; Nakabayashi et al., 1992; Van Meerbeek et al., 1992a, 1993a; Eick et al., 1993b; Walshaw and McComb, 1994).

Several current dentin adhesive systems use the wetting agent 2-hydroxyethylmethacrylate (HEMA), and it has been demonstrated that when HEMA is applied to the conditioned dentin surface, bond strengths are enhanced (Munksgaard et al., 1985; Erickson, 1989, 1992; Nakabayashi and Takarada, 1992). The morphology of the dentin adhesive interface has been observed by scanning electron microscopy (SEM) (Chappell et al., 1991; Gwinnett and Kanca, 1992; Nakabayashi, 1992; Van Meerbeek et al., 1992a, 1993b; Eick et al., 1993b; Pashley et al., 1993; Chappell and Eick, 1994; Walshaw and McComb, 1994) and transmission electron microscopy (TEM) (Eick et al., 1991, 1992, 1993a,b; Eick, 1992; Nakabayashi, 1992; Nakabayashi et al., 1992; Van Meerbeek et al., 1993a).
Other investigators have attempted to determine the chemical nature of the dentin adhesive interface with analyses by x-ray photoelectron spectroscopy (XPS or ESCA) (Eliaades et al., 1990), Fourier transform infrared (FTIR) spectroscopy (Stangel et al., 1991; Spencer et al., 1992), and micro-Raman spectroscopy (Suzuki et al., 1991; Van Meerbeek et al., 1993b). Although there is some controversy in the literature, the present chemical analysis techniques strongly suggest that the nature of the adhesive bond is mechanical and results from the formation of a hybrid layer, in which the adhesive resin penetrates the interfibrillar spaces of the collagen meshwork exposed by the pre-treatment (Nakabayashi, 1992; Eick et al., 1993a,b; Pashley et al., 1993; Van Meerbeek et al., 1993a,b). Only the study by Van Meerbeek et al. (1993b), that combined micro-Raman analysis with SEM, attempted to compare the morphology of the dentin adhesive interface with its chemistry. More complete information is needed on how the adhesive resin penetrates and interacts with the demineralized layer so that we might more fully understand the nature of the dentin adhesive bond and thereby potentially improve the performance of dentin adhesives.

The purpose of this investigation was to correlate the location of an adhesive wetting agent (HEMA) directly with the morphology of the demineralized layer produced by bonding systems with various dentin pre-treatments. Scanning transmission electron microscopy (STEM) and energy-dispersive spectroscopy (EDS) were performed on the same ultrathin sections. We modified HEMA by replacing an oxygen atom on its structural backbone with a sulfur atom (thiolated HEMA or 2-hydroxyethylthiomethacrylate; HETMA) to permit elemental identification of the adhesive wetting agent using EDS.

Materials and methods

Freshly extracted human impacted third molars were stored in normal saline solution at 4°C immediately following removal and prepared within 2 days of surgery. The occlusal one-third of each tooth was removed perpendicular to the long axis of the tooth with a diamond saw (Isomet Low Speed Bone Saw; Buehler Ltd., Lake Bluff, IL), with room-temperature distilled water used as a coolant. The dentin surface of the apical section was then surfaced through 320 grit with wet silicon carbide paper, producing a smear layer. Next, the etchant or primer treatment for each of four adhesive systems (A, 10% citric acid and 3% ferric chloride; B, 10% maleic acid; C, 2.5% wt/wt nitric acid; or D, HEMA and an alcoholic solution of phosphorus acid ester [PENTA]) was applied per manufacturers’ directions to individual teeth.

A 10% solution (vol/vol) of HETMA in acetone was applied to each conditioned or primed dentin surface and dried. Three teeth were prepared for each adhesive system. The polymerization of the HETMA was not initiated prior to application of the appropriate adhesive. Following the application of HETMA, each adhesive was applied and activated following the manufacturers’ directions.

The teeth were then dehydrated, infiltrated, embedded, and sectioned, as described previously (Eick et al., 1991, 1992, 1993a,b; Eick, 1992), for STEM observation and analysis. Three-hundred-nanometer-thick sections were cut for STEM/EDS analysis. They were not stained, nor had the source specimens been osmicated before embedment. Separate 90-nm-thick sections of dentin adhesive systems A, B, and C were prepared from the same non-osmicated specimens, stained with uranyl acetate and lead citrate, and photographed in TEM mode to provide better detail of the dentin adhesive interfacial region. Of the thicker, unstained sections, over 20 specimens of each test condition were viewed through a Philips (Mahwah, NJ) CM12 scanning transmission electron microscope equipped with an EDAX (Mahwah, NJ) 9900 EDS system. Brightfield STEM images of the dentin adhesive interfacial region of each system were collected at 10,000x and 17,500x; EDS elemental area scans of calcium, phosphorus, sodium, chlorine, sulfur, oxygen, carbon, and silicon were taken simultaneously. For brevity, only one of these magnifications per adhesive system is included in this report.

Results

Three separate HEMA analogues were made—two in which the methyl group of the HEMA molecule was replaced by either a chlorine or a bromine atom and another in which the internal oxygen atom was replaced by a sulfur atom. The sulfur-substituted material (HETMA) produced the highest peak profiles above background for EDS analysis, and its physical and wetting properties were very similar to those of HEMA. (Patent application procedures are currently being followed for HETMA and preclude disclosure of the details of its synthesis at this time.) Solubility parameter and molar refractivity values for HETMA and HEMA are listed in the Table.

After preparing 90-, 300-, and 500-nm-thick non-demineralized sections, we determined that those sections 300 nm thick produced the best specimens for dual analysis by EDS and photomicrography.

No photomicrographs were taken of 90-nm-thick sections of adhesive system D, using a dentin pre-treatment of HEMA and PENTA; there was no demineralized layer produced when this system was used.

System A, using a dentin pre-treatment of 10% citric acid and 3% ferric chloride, is illustrated in Fig. 1. The hybrid layer (delineated by arrows) runs from left to right in the upper one-third of the images. There is some dentin debris,

### Table. Comparison of hydroxyethylthiometacrylate (HETMA) and 2-hydroxyethylmethacrylate (HEMA)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HEMA</th>
<th>HETMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility (Joules/cc)1/2</td>
<td>23.6 ± 1.4</td>
<td>25.0 ± 9</td>
</tr>
<tr>
<td>Molar Refractivity (calculated from the Lorentz-Lorenz equation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated</td>
<td>34.0</td>
<td>37.4</td>
</tr>
<tr>
<td>Theoretical</td>
<td>32.6</td>
<td>37.1</td>
</tr>
</tbody>
</table>

1 Van Krevelen (1990).
probably remaining from the smear layer, or reprecipitated calcium phosphate, that lies immediately above the hybrid layer. It can be seen that the HETMA penetrated the demineralized layer completely and was in intimate contact with the underlying nondemineralized dentin surface. The concentration of HETMA appears to have been fairly uniform throughout the hybrid layer (Figs. 1A, 1B). The same sample, sectioned at 90 nm, is shown in Fig. 2; arrows indicate examples of banded collagen.

In the bonding system incorporating 10% maleic acid as a dentin pre-treatment (system B; Fig. 3), the hybrid layer (arrows) runs from left to right in the upper half of the images. Banded collagen remained in the layer of dentin demineralized by the pre-treatment (Fig. 4, arrows), and the HETMA again appears to have completely penetrated the demineralized layer and come into contact with the underlying nondemineralized dentin (Fig. 3B). In addition, HETMA filled the dentinal tubules that lie below the hybrid layer (Fig. 3B).

A different pattern of HETMA penetration was observed when the dentin was treated with nitric acid (system C; Fig. 5). The demineralized layer runs from left to right in the upper one-third of the images in Figs. 5A (see arrows) and 5B. Two dentinal tubules, cut longitudinally, extend perpendicular to the demineralized layer from the middle third to the bottom of the images. HETMA has filled these tubules and wetted the outermost portion of the demineralized layer, but it has not penetrated entirely through this layer to the underlying nondemineralized dentin (see Fig. 5B). From the same specimen, at 90-nm thickness and stained with uranyl acetate and lead citrate, a narrow band of electron-dense material runs diagonally through the lower half of the image (Fig. 6). This band clearly delineates the original edge of the tooth, separating the outer portion of the demineralized layer from the adhesive resin. Banded collagen can be demonstrated in the demineralized layer of these samples (Fig. 6). The presence of banding is more apparent in proximity to the nondemineralized dentin (Fig. 6, arrows). From these results, it would appear that the nitric acid pre-treatment has denatured the collagen in the outer portion of the demineralized layer. The thickness of the demineralized layer in the nitric-acid-treated specimens was always less than that seen in specimens treated with other bonding systems (approximately 1 vs. 2 to 3 μm in these specimens; see Figs. 2, 4, and 6).

The combination of HEMA and PENTA, comprising the primer for system D, slightly altered and removed the smear layer but did not produce a wide demineralized layer when compared with the other three adhesive systems (Fig. 7A). The 10% HETMA applied following the primer does not appear to have concentrated at the surface of the preparation; it does appear, however, to be in intimate contact.
contact with the dentin or modified smear layer. As with the other adhesive systems, HETMA has entered the dentinal tubules (Fig. 7B).

Discussion

To be able to trace the movement of HEMA (which contains only carbon, hydrogen, and oxygen) following its application to a treated dentin surface, we "tagged" the HEMA molecule with an identifying element that would lend itself to EDS analysis. Thiolated HEMA (HETMA) was developed for this purpose.

The development and use of HETMA allowed a direct correlation to be drawn between the morphology of the demineralized layer and the penetration and exact location of a wetting agent. Previously, only the micro-Raman spectroscopy study by Van Meerbeek et al. (1993b) had attempted to correlate the distribution of an adhesive/wetting agent with the microstructure of the hybrid layer. An advantage of the micro-Raman technique (Suzuki et al., 1991; Van Meerbeek et al., 1993b) is that a spectrum is produced in which molecular groups can be identified. Therefore, it is not necessary to "tag" or identify a specific element, as was the case with the EDS analysis used in the present study.

The resolution of the micro-Raman technique, however, is approximately 1 μm, and it is difficult to correlate the morphology to the exact area of chemical analysis. An advantage of the STEM system with EDS is that high-resolution transmission electron micrographs can be obtained, and elemental maps of the same specific area can be analyzed. The resolution of the present STEM/EDS configuration is on the order of tens of nanometers, far superior to that of the micro-Raman technique. The EDS system does require a specific element characteristic of the material of interest, in this case sulfur, to be present. Fortunately, HEMA could be modified to HETMA with the substitution of a sulfur atom for an oxygen atom along the backbone of its molecular structure, and the characteristics of HETMA, especially its wetting behavior (see Table), are very similar to those of HEMA. Thus, HETMA served as a very adequate substitute for HEMA and permitted the exact location of the wetting agent to be determined in relation to the demineralized layer.

The application of 10% HETMA in acetone following the dentin pre-treatment represents a departure from the methodology used by each manufacturer. HEMA, however, is a component of two of these adhesive systems, and the use of its analogue at this early step facilitated a controlled exploration of the differences among the dentin pre-treatments. Acetone is simply the solvent carrier for the HETMA; it is thought to evaporate rapidly, leaving 100% HETMA in the region of interest (Robert Erickson, personal communication).

A knowledge of how the adhesive interacts with the dentin surface and what influence the structure of the dentin surface has on the wetting behavior of the adhesive...
Figure 5. The system C adhesive, utilizing a dentin pre-treatment of 2.5% nitric acid, plus 10% HETMA, sectioned at 300 nm, and photographed and analyzed without heavy metal stains. (A, left) Transmission electron photomicrograph at 10,000x original magnification. Arrows delineate the demineralized zone, which is ≤ 1 μm thick. DT, dentinal tubule; bar = 1 μm. (B, right) Elemental concentration map of the area shown in Fig. 5A. Cyan represents phosphorus; red represents sulfur.

Figure 6. Transmission electron micrograph of the specimen shown in Fig. 5 sectioned at 90 nm and stained with uranyl acetate and lead citrate. Arrows indicate banded collagen; bar = 1 μm.

is essential to an understanding of the behavior of the adhesive bond. It has been suggested (Nakabayashi, 1992; Eick et al., 1993b; Pashley et al., 1993; Van Meerbeek et al., 1993a) that the structure of the demineralized layer, specifically the presence of banded collagen in this layer following acid pre-treatment, increases the bond strength (Eick et al., 1993a,b). The two adhesives using either 10% citric acid/3% ferric chloride or 10% maleic acid dentin pre-treatment have shown excellent shear bond strength values in other studies (Barkmeier and Cooley, 1991; Chappell et al., 1991; Eick et al., 1993a,b; Chappell and Eick, 1994). In the present study, banded collagen was present in the hybrid layers that were formed when either of these two materials was used. Of potentially greater importance was the penetration of the HETMA throughout the demineralized layer. Apparently neither of these dentin pre-treatments denatures the collagen enough to create a

Figure 7. The dentin adhesive (system D) with a pre-treatment of HEMA and PENTA, plus 10% HETMA, sectioned at 300 nm and left unstained. (A, left) Transmission electron photomicrograph at 10,000x original magnification. DT, dentinal tubule; bar = 1 μm. (B, right) Elemental concentration map of the area shown in Fig. 7A. Cyan represents phosphorus; red represents sulfur.
gel that might interfere with the penetration of the adhesive wetting agent. The formation of an impermeable gel, due to acid pre-treatment of the dentin surface or smear layer remnants, has been suggested by Pashley et al. (1993) and by Eick et al. (1993a) as a possible inhibiting factor in the development of an excellent adhesive bond. The lack of penetration of HETMA into nitric-acid-treated dentin would support the conclusion that a collagenous gel inhibited the penetration of the adhesive into the demineralized layer. An alternative explanation might be that the nitric acid treatment permitted the demineralized collagen to collapse, thereby reducing its porosity (Pashley et al., 1993) and hence the ability of the HETMA to penetrate. This was reflected in a more narrow demineralized layer compared with those created when citric acid/ferric chloride or maleic acid was used. Perhaps the original demineralized layer was 2 to 3 μm thick but collapsed to about 1 μm thick. The structural characteristics of the demineralized layer were apparently preserved by the use of 10% maleic acid, as reflected in the excellent HETMA penetration (Pashley, personal communication).

Shear bond strengths for the system using the nitric acid pre-treatment were only 12 to 14 MPa, whereas those for the systems using citric acid/ferric chloride or maleic acid were from 17 to 22 MPa (Chappell et al., 1991; Chappell and Eick, 1994).

Apparently, the presence of a demineralized layer that is adequately penetrated by the adhesive is also needed for adequate bond strengths (> 17 MPa). The system using the HEMA/PENTA primer did not produce a demineralized layer; its bond strength was between 12 and 14 MPa (Chappell and Eick, 1994). Again, these results strongly suggest that a demineralized layer that maintains the integrity of the banded collagen, and a wetting agent that fully penetrates this zone, are necessary for good adhesive bonds, at least with currently available dentin adhesive systems.

In summary, the combined STEM and EDS system used in this study permitted a correlation between the morphology of the dentin surface and the wetting and penetration characteristics of a commonly used wetting agent. It is suggested that the presence of intact collagen, the lack of an impermeable gel of denatured collagen, and the complete penetration of the wetting agent are essential requirements for the successful behavior of present-day dentin adhesives. The use of STEM and EDS analysis, as in the present study, should contribute significantly to future improvements of dentin adhesive systems, especially when these results are supported by data from other techniques, such as photoacoustic FTIR, and micro-Raman and Auger spectroscopy.

Acknowledgments

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