The Nanoleakage Patterns of Different Dentin Adhesive Systems

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I. Introduction

Previous studies have focused on marginal gaps as the major pathway of microleakage. But Sano et al. described the occurrence of microleakage in the absence of gaps and the subsurface porosity of demineralized dentin. Yu and Davis described microleakage within or beneath smear layers and resin-bonded dentin using silver nitrate and SEM/EDS techniques. These results indicate that investigators should examine microleakage pathways at high magnification rather than simply relying on macroscopic dye penetration.

Dentin adhesive systems with their micro-

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II. Materials and Methods

Thirty freshly extracted human molars stored in 0.5% chloramine-T solution were used. Occlusal, buccal and lingual enamel was removed using a model trimer under running water. The flat occlusal dentin surfaces were examined under a microscope to insure that no enamel remnants remained. The dentin surfaces were wet ground with 500-grit silicon carbide paper (Buehler Ltd., Lake Bluf, IL, USA) for 60 sec.

Three dentin adhesive systems were used in this study (Table 1). Scotchbond Multi-Purpose (MP) was used as total etching adhesive system. Clearfil SE Bond (SE) and Prompt L-pop (LP) were used as self-etching adhesives. Each 10 dentin surfaces were treated with one of the dentin adhesive systems following each manufacturer’s instruction (Table 2). 2mm thick of Z-250 hybrid resin composite (3M Dental Products Inc., St. Paul, USA) was light-cured for 40 sec using XL 3000 curing light (3M) on the treated surface. After storage in tap water at 37°C for 24 hr, the margins were finished and polished with Soflex disks (3M). The specimens were checked using a microscope to ensure that no flash was left along the exposed dentin surfaces.

Five teeth of each dentin adhesive group were thermocycled at 5-55°C water for 30,000 cycles. Dwell time was 1 min and transfer time was 15 sec for each cycle. Root apices of all teeth were sealed with Z-250 composite (3M), and the entire tooth surface except for the bonded interface and 1mm of the tooth surface adjacent to the interface, was coated with two layers of nail varnish. The teeth were placed in 50% (w/v) silver nitrate solution in total darkness for 24 hr, rinsed in running water for 5 min, and immersed in photodeveloping solution under fluorescent light for 8 hr in order to reduce the silver ions to metallic silver. After removal from the developing solution, the teeth were placed in running water for 5 min. The teeth were embedded in translucent resin (Epofix: Struers, Denmark), and sectioned buccolingually across the bonded surface, making a total

The purpose of this study was: (1) to compare nanoleakage patterns of total etching system and self-etching adhesives and (2) to investigate the change of the nanoleakage patterns after thermocycling.
Table 1. Materials, manufacturers and compositions

<table>
<thead>
<tr>
<th>Adhesive system</th>
<th>Etchant</th>
<th>Primer</th>
<th>Adhesive resin</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotchbond Multi-Purpose</td>
<td>35% H3PO4</td>
<td>HEMA, light-cured polymer, water</td>
<td>Bis-GMA, HEMA, photoinitiator</td>
<td>3M Dental Products Inc., St. Paul, USA</td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>Self-etching primer</td>
<td></td>
<td>MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, water</td>
<td>Kuraray Co., Ltd., Osaka, Japan</td>
</tr>
<tr>
<td>Prompt</td>
<td>One-step</td>
<td></td>
<td></td>
<td>3M-ESPE, Germany</td>
</tr>
<tr>
<td>L-Pop</td>
<td>Water, stabilizer, parabens: methacrylated phosphoric acid eaters: fluoride complex: photoinitiator (BAPO)</td>
<td></td>
<td>Seefeld, Germany</td>
<td></td>
</tr>
</tbody>
</table>

BAPO, bis-acryl phosphate oxide; HEMA, hydroxyethylmethacrylate; Bis-GMA, bisphenol glycidyl dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphotetate

Table 2. Bonding procedures according to the manufacturer’s instructions.

<table>
<thead>
<tr>
<th>Adhesive system</th>
<th>Etching</th>
<th>Priming</th>
<th>Bonding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotchbond Multi-Purpose</td>
<td>apply 15 sec, rinse 10 sec, dry gently</td>
<td>apply primer for 20 sec, air dry mildly</td>
<td>apply adhesive, air thin gently, light-cure for 20 sec</td>
</tr>
<tr>
<td>Clearfil SE Bond</td>
<td>Self-etching priming: apply with a brush, leave undisturbed for 20 sec, air dry mildly</td>
<td>Bonding: apply with a brush, air thin gently, light-cure 10 sec</td>
<td></td>
</tr>
<tr>
<td>Prompt</td>
<td>Apply with continuous scrubbing motion for 15 sec, gently air dry, light cure for 10 sec</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of 20 specimens for each dentin adhesive system.

All the sectioned surfaces were polished with increasingly fine diamond pastes (6, 3, 1 μm; Buehler Ltd., Lake Bluf, IL, USA). The specimens were cleaned ultra-sonically, air dried, mounted on aluminum stubs and placed in a desiccator for 24 hr, and coated with thin gold. 10 specimens for each group were examined under scanning electron microscope (SEM: JSM840A Jeol, Japan) in backscattering electron mode. The silver particles were confirmed with energy dispersive X-ray system (EDS: Oxford, England).

III. Results

All specimens in the six groups showed nanoleakage at the adhesive/dentin interface. Seven specimens (2 in MP, 2 in SE, 3 in LP thermocycled groups) were excluded from the analysis because of definite marginal micr leakage as visible gaps between dentin and composite (Fig. 2(a)). The silver particles were confirmed with EDS (Fig. 2(b)). The conical shape of global silver penetration pattern was shown in Fig. 1(a). And the gap free margins are illustrated in Fig. 1(b). Typical leakage patterns at the adhesive/dentin interfaces for each dentin adhesive system are illustrated in Fig. 2(a)-(d) under the SEM observation.

Scotchbond Multi-Purpose produced a very distinct hybrid layer which was approximately 15-20 μm thick. Distinct resin tags were also observed in Fig. 3(a). Accumulation of silver particles were noted at the base of the hybrid layer. And in some parts, loosely distributed patches of silver
deposite appeared in the hybrid layer. Silver was also deposited around the resin tags. Peri and lateral tubules were filled with silver.

The leakage patterns for Clearfil SE Bond was illustrated in Fig. 3(b). The hybrid layer was approximately 2-3μm thick. The resin tags were not clear. Silver particles were deposited along the lower half of the hybrid layer by thin line. At top of the hybrid layer, amorphous silver particles were also identified beneath the layer of composite resin.

Prompt L-Pop produced a thin hybrid layer of approximately 2-3μm (Fig. 3(c) and (d)). This system showed few resin tags. Silver deposited along the base of the hybrid layer, with isolated deposits located throughout its whole thickness in many specimens. In another specimens, distinct bands of silver deposits could be detected within the hybrid layer, but silver deposition was not distinct in the base of the hybrid layer.

There were no changes in leakage patterns in thermocycled groups. But the silver distribution in the base of and within the hybrid layer was somewhat more intense (Fig. 4(a)-(d)).
Fig. 3. The nanoleakage patterns: (a) Scotchbond Multi-Purpose, (b) Clearfil SE Bond, (c) and (d) Prompt L-Pop. (a) shows silver accumulation at the base of hybrid layer. In (b), thin line of silver was noted in the base of hybrid layer. And distinct bands of silver deposits were observed within the hybrid layer in (c) and (d). C: composite resin, D: demineralized dentin.

Fig. 4. The nanoleakage patterns of thermocycled groups: (a) Scotchbond Multi-Purpose, (b) Clearfil SE Bond, (c) and (d) Prompt L-Pop. Note that there were no differences in nanoleakage patterns between Fig. 3(a)–(d) and Fig. 4(a)–(d) except more intense silver penetration in Fig. 4(a)–(d). C: composite resin, D: demineralized dentin.
IV. Discussion

In general, all dentin adhesive systems used in this study showed nanoleakage within the hybrid layer and adhesive resin to some extent. This may be a consequence of incomplete penetration of the adhesive resin into the demineralized dentin, leaving the collagen not enveloped by resin, or this may result from polymerization shrinkage. The nanoleakage pathway may be located within the adhesive resin, within the hybrid layer and within partially- or fully-demineralized dentin. Porosity in the hybrid layer not infiltrated by adhesive resin is the most important pathway for nanoleakage and influences the durability of the bond, since solutions can penetrate into these nanometer-sized spaces and hydrolyze the exposed collagen and adhesive resin. The spaces are too small to allow bacterial immigration. However, bacterial products, e.g. acids, might use nanoleakage as penetration pathways through the hybrid layer and dentinal tubules. And the unprotected protein at the dentin adhesive interface may be accessible to bacterial proteolytic enzyme.

The silver crystals have a minimum diameter of 3 to 5 nm; after longer treatment these small crystals fuse to form large aggregates of 15 to 50 nm diameter. Collagen can be stained strongly with silver. The resin of current dentin adhesive systems has been reported not to permeate uniformly within the spaces around the collagen fibrils, leaving them exposed. It may be speculated that when the specimens were immersed in the silver nitrate solutions, the silver penetrated the spaces existing within the hybrid layer by seeping around and precipitating onto the exposed collagen fibrils.

In the dye penetration studies reported in the literatures, the thermocycling regimens varied in temperature number of cycles and dwell time. Temperature extremes have varied among researchers with a mean low temperature of 6.6°C and mean high temperature of 55.5°C. The time of immersion of specimens in hot or cold fluids has varied from a few seconds to several minutes. Harper et al. pointed out that a patient would not tolerate direct contact of vital tooth with extremely hot or cold substances for extended periods of time, so dwell time longer than 15 sec would seem to be inappropriate. According to the study of Miyazaki et al., the bond strength of dentin adhesive systems decreased after 30,000 cycles, especially in the self-etching systems. Thus, temperature of 5-55°C, dwell time of 15 sec and 30,000 cycling numbers were selected in this study.

During the thermal cycling test, the hot water may accelerate hydrolysis of the resin composite as well as the bonding agent and extract poorly polymerized resin oligomers. The results of this study showed that thermocycling did not affect nanoleakage greatly for dentin adhesive systems used. The results are in agreement with those of previous microleakage studies. Harper et al. showed that the rate of temperature change beneath resin restorations was relatively low after taking cold or hot fluids, indicating that the linear coefficient of thermal expansion would not greatly influence the dimensional change of the material and therefore not affect microleakage. Stryom et al. suggested that the bonding of the adhesive to tooth structure is micro-mechanical in nature and would not be adversely affected by temperature cycling. High bond strength and the formation of a hybrid layer at the adhesive/dentin interface resulted in gap-free restorations. Although porosities are still present within the hybrid layer and may permit molecular leakage, it is not believed that thermal stresses imposed on the restorations would be great enough to enlarge the porosities or create gaps at the interface and subsequently increase the dye penetration. The nanoleakage phenomenon is related to the process of bonding and is not caused by a later fatigue of the hybrid layer.

The bonding agents used in this study achieved almost totally gap-free margins at the dentin-restoration interfaces. This may be attributed to several factors. The stress generated by polymerization contraction of resin composites has been reported to be about 13-17 MPa and shear bond
strength of approximately 21 MPa is believed to be necessary to prevent marginal contraction gaps\textsuperscript{30}. All three adhesive systems in this study are capable of achieving higher bond strengths, which theoretically resist the polymerization contraction stresses. Feilzer et al.\textsuperscript{31} found that the resulting stress inside a restoration was proportional to the ratio of its bonded to non-bonded surfaces. The flat occlusal composite restorations in this study have almost as much free surface area as bonded surface area, and hence have a C-factor of less than 1. Feilzer et al.\textsuperscript{27} stated that restorations with C\textsubscript{1} are the only restorations likely to survive polymerization contraction stresses. All these factors may explain the gap-free margins observed in this study, even though bulk placement technique was employed. The composite thickness was less than 2mm, which is the recommended initial thickness for the incremental technique. Using silver nitrate staining, the artificial gaps created upon high vacuum dehydration during specimen preparation can be easily differentiated from true gaps by the absence of silver staining along the gap border with the backscattered electron mode\textsuperscript{28}. The conical shape of the penetration-induced structure can be explained by the diffusion behavior (Fick’s first law) of the silver nitrate solution during the exposure time of 24 hr\textsuperscript{30}.

Scotchbond Multi-Purpose demonstrated more intense accumulation of silver in the base of hybrid layer than Clearfil SE bond and Prompt L-Pop. This may mean that Scotchbond Multi-Purpose has much more porosity in the base of hybrid layer than other systems. Silver ions are easily reduced to metallic silver by organic material, especially in the presence of collagen fibers. Intense silver accumulation along the base of hybrid layer may have resulted from that silver ions had filled collagen-rich and resin-poor spaces at the bottom half of demineralized dentin\textsuperscript{30}. Silver deposition in the hybrid layer may represent incomplete removal of water. Using air stream to dry applied primer may not effectively remove residual water from a HEMA/water primer\textsuperscript{31}. Remaining water interrupts the formation of adequate polymer network, leaving silver penetrable porosity.

Clearfil SE Bond simultaneously etches and primes the dentin, allowing the adhesive resin to fully penetrate the demineralized dentin. So theoretically silver deposition may not occur at the base of hybrid layer. However, in this study, this system could not ensure complete penetration of adhesive resin into the inter-fibril collagen spaces, otherwise, silver would not appear within the hybrid layer.

Prompt L-Pop demonstrated minimal deposition of silver in the base of hybrid layer and the distributed silver particles in the whole hybrid layer. This nanoleakage patterns cannot be attributed to incomplete resin infiltration. These micro-voids more likely represent areas in which water was incompletely removed from the resin-dentin interfaces. The presence of residual water within the adhesive may lead to domains of incomplete polymerization of the adhesive or sequestrations of more hydrophilic oligomers in these particular regions\textsuperscript{30}. All self-etch systems contain water to ionize the acidic monomers for effective demineralization of dentinal hard tissues\textsuperscript{9}. Whereas water is comparatively easy to remove with the use of acetone-based adhesives, because its azeotrope contains more water and it has a higher vapor pressure, it is difficult to remove the last traces of water from ethanol-based adhesives, due to the increased capacity of ethanol to form hydrogen bonds with water\textsuperscript{9}.

The deeper the demineralized dentin and thicker the hybrid layer, the greater is the potential for nanoleakage of silver ions within the partially- or fully-demineralized dentin and the hybrid layer, or within partially polymerized adhesive resin\textsuperscript{31}. The critical index of the quality of a hybrid layer may be how much silver it takes up per unit volume or per unit depth. Self-etching systems that do not etch as deeply as total etch systems may have as much or more silver uptake per unit volume of hybrid layer than more aggressive etching systems\textsuperscript{31}.

Although the mechanisms leading to the nanoleakage phenomenon are not completely
explored, it seems to be evident from our study that nanoleakage is mainly due to a discrepancy between the depth of etching and the depth of resin penetration in the cases of total etching systems like Scotchbond Multi-Purpose. Also, it should be considered that non-polymerized primer monomers and residual water additionally might affect pores in the hybrid layer region which form nanoleakage as well in the cases of self-etching systems like Clearfil SE Bond and Prompt L-Pop. It is too early to judge the clinical relevance of nanoleakage. But the influences in marginal discoloration, recurrent caries, post-operative symptoms and the longevity of the composite restoration cannot be excluded. Further studies are needed to evaluate the clinical significance of this phenomenon and, if necessary, to develop adhesive systems minimizing nanoleakage in order to optimize dentin bonding.

V. Conclusions

1. Different nanoleakage patterns were observed with the different adhesive systems.
   - In the group of MP, accumulation of silver particles were noted at the base of the hybrid layer. And in some parts, loosely distributed patches of silver particles appeared in the hybrid layer. Silver was also deposited around the resin tags, the peri and lateral tubules.
   - In the group of SE, silver particles were deposited along the lower half of the hybrid layer by thin line. At top of the hybrid layer, amorphous silver particles were also identified beneath the layer of composite resin.
   - In the group of LP, silver deposited along the base of the hybrid layer, with isolated deposits located throughout its whole thickness in many specimens. In another specimens, distinct bands of silver deposits could be detected within the hybrid layer, but silver deposition was not distinct in the base of hybrid layer.
   - The dentin adhesive systems used in this study did not achieve perfect sealing at the compos-

3. There were no changes in nanoleakage patterns in thermocycled groups. But the silver distribution in the base of and within the hybrid layer was somewhat more intense.

References

17. Gale MS and Darvell BW: Controlling dentin penetration in computer micro-leakage tracer mapping. J Dent