Interface Characterization and Nanoleakage of One-step Self-etch Adhesive Systems

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Abstract

Purpose: To evaluate the performance of 2 one-step self-etch adhesive systems and a 1 two-step etch-and-rinse (as a control) by examining the resin-dentin interface and assessing the nanoleakage.

Materials and Methods: Cervical class V cavities were prepared in 30 sound human premolars. Two one-step, G-Bond (GB) and Adper Prompt L-Pop (APL), and 1 two-step, Excite (Ex), adhesive systems were used to bond the composite restorations. The restored teeth were incubated in water at 37±1°C for 24 h. Fifteen were subjected to further immersion in 50% ammoniacal silver nitrate solution for 24 h and soaked in photo-developing solution for 8 h. All teeth were sectioned across the bonding surface in bucco-lingual direction. The cut surfaces were polished, followed by 6N HCL application for 45s. All surfaces were metal coated before conducting the ultra-morphological observation, using the Field Emission Scanning Electron Microscope (FE-SEM), to characterize the hybrid layer and evaluate the nanoleakage.

Results: A significant difference in the thickness of the hybrid layer was evident between all adhesive systems (ANOVA, p<0.05). A thin transitional zone about 0.75 (0.16) µm wide was found at the interface of GB. Whereas APL and Ex showed wider interaction layers nearly 1.65 (0.23) and 2.22 (0.23) µm. GB did show a minimal degree of nanoleakage in comparison to that shown in case of either APL or Ex.

Conclusion: GB provides a nano-interaction layer at the dentin/adhesive interface that seems to play an important role in minimizing the degree of associated nanoleakage.

INTRODUCTION

Although gap-free margins at the dentin/restoration interface could be achieved with some adhesive systems utilizing the “total-etch” technique, Sano et al., have described another pattern of leakage, by observing the penetration of silver nitrate along gap-free margins by the aid of either scanning (SEM) or transmission electron microscopy (TEM). Since leakage was found to occur within the nanometer-sized spaces around the collagen fibrils within the hybrid layer, it has been termed “nanoleakage”.

The nanoleakage usually represents permeation laterally through the hybrid layer and could be the result of the incomplete infiltration of adhesive resin into the demineralized dentin. This kind of leakage may also allow the penetration of bacterial products or oral fluids along the interface, which may result in hydrolytic breakdown of either the adhesive resin or collagen within the hybrid layer, thereby compromising the stability of the resin-dentin bond.

In spite of the technique-associated difficulties, previous studies indicated the efficiency of 50% silver nitrate solution in detecting nanoleakage within the hybrid layer. However, the acidity of that solution (pH = 3.4-4.5) during long time immersion could induce demineralization around the edges of the restorations, particularly into the partially-demineralized dentin, leading to false positive results. The modified silver-staining technique was recently suggested to overcome the drawback of silver nitrate solution. It utilizes 50wt% ammoniacal silver nitrate having pH measured at 9.5 aiming to eliminate the possibility of...
dentin dissolution at the hybrid layer. Although the nanoleakage for various adhesive systems and its influence on bond strength have been evaluated in previous studies, this in vitro study aimed to evaluate the thickness of the interaction layer and nanoleakage of 2 recently-introduced one-step adhesive systems and 1 two-step etch-and-rinse (as a control).

The null hypothesis tested was that the one-step adhesive system (G-Bond) might offer better adhesion to cavity walls via formation of a nano-interaction zone instead of the traditional hybrid layer.

**MATERIALS AND METHODS**

Thirty sound freshly extracted premolars of patients indicated for orthodontic treatment were collected at the Department of Oral Surgery, Faculty of Dentistry in both Mansoura and Suez Canal Universities. The collected teeth were cleaned using the Pro-sonic 300 MTH (Sultan Chemists, Englewood, NJ, USA), and examined to ensure the absence of cracks using a binocular microscope at 20x magnification (Olympus Co., Tokyo, Japan). The selected teeth were stored in deionized water that contained antibacterial agent (0.2% sodium azide) for a maximum of 1 month. Using No. 330 bur (Brasseler USA, Savannah, GA, USA) at high speed with copious air/water spray, standardized 2 mm deep class V cavities were prepared at the buccal surfaces 0.5 mm coronal to the cement-enamel junction of all teeth. The prepared margins were all placed in enamel. A metal, buccal-fitted, index was used to guide the external cavity outlines and a rubber stopper hanged to the bur’s shank has aided in determining the accurate cavity depth. All the prepared cavities were thoroughly rinsed with water and air-dried before receiving the composite restorations. Table 1 shows the description and manufacturers of materials used in this study.

Table 1: Materials used in this study

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-Bond (GB)</td>
<td>Self-etch (no mix) adhesive</td>
<td>phosphoric acid ester monomers, UDMA, 4-MET, TEGDMA, photoinitiators, water, initiators</td>
<td>3M ESPE Dental Products, St Paul, MN, USA</td>
</tr>
<tr>
<td>Adper Prompt L-Pop (APL)</td>
<td>Self-etch (no mix) adhesive</td>
<td>Composant 1: Methacrylate phosphoric acid ester, photoinitiators, stabilizers</td>
<td>3M ESPE Dental Products, St Paul, MN, USA</td>
</tr>
<tr>
<td>Excite (Ex)</td>
<td>Etch and mix adhesive</td>
<td>HEMA, DMA, phosphoric acid acrylate, highly deoxygenated water, initiators and stabilizers in alcohol solution</td>
<td>Procter &amp; Gamble Plano, TX, USA</td>
</tr>
<tr>
<td>Tetric Ceram</td>
<td>Light-curing, fine-particle hybrid resin composite</td>
<td>BisGMA, UDMA, TEGDMA, methyl methacrylate, barium glass, pigments and initiators</td>
<td>Ivoclar Vivadent, Schaan, Liechtenstein</td>
</tr>
<tr>
<td>Silica matrix crystals</td>
<td>Silica matrix crystals</td>
<td>Sigma Chemical Co. St. Louis, MO, USA</td>
<td></td>
</tr>
<tr>
<td>2% Ammonium hydroxide solution</td>
<td>2% Ammonium hydroxide solution</td>
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</table>

Two one-step, self-etch (G-Bond); and (Adper Prompt L-Pop) adhesive systems were used to retain the Tetric Ceram hybrid composite restorations. In addition, 1 two-step, etch-and-rinse (Excite) adhesive system was also selected to serve as control (n= 10 cavities for each). The self-etch adhesive systems were applied to the prepared cavities and left for 10s before air thinning. Curing of both self-etching adhesives was performed by the use of a light-curing device (Hawe-Neos, Gentilino, Switzerland) for 10s. The dentin surfaces of control cavities were etched with 37% phosphoric acid (Ivoclar-Vivadent) for 15s, washed under copious air-water spray, then the excess water was removed by blotting with a tissue paper leaving the dentin visibly moist. The Excite adhesive was then applied using a brush for 10s and light-cured for 20s after air-drying of the excess material. The cavities were then filled with composite, and cured for 40s before finishing using 1000-grit abrasive paper (SAITAC Abrasive, Torino, Italy) in presence of water-cooling. The restored teeth were incubated in distilled water at 37±1°C for 24 h before sectioning and testing have taken place.

**ASSESSING THE DENTIN/ADHESIVE INTERFACE**

Five premolars from each group were half-sectioned buccolingually across the bonding surface with a low-speed diamond saw (model 650, South Bay Technology Inc., CA, USA). The cut surfaces were then lightly finished using a sequence of 600, 800, and 1000-grit abrasive papers (SAITAC Abrasive) in presence of water-cooling followed...
with light polishing with diamond paste. The samples were then immersed in 6N (mol/L) HCL for 45 seconds then rinsed with distilled water and dried for 24 h at 37°C (Binder Incubator, Type B28, Tuttlingen, Germany). The dried samples were sputter coated with gold-palladium (JEOL, JFC, 1100e, Tokyo, Japan.) The dentin/adhesive interface of each sample was then examined using a field emission scanning electron microscope (FE-SEM) at 30 KV (JEOL, JSM, 5600LV, Tokyo, Japan). The thickness of interaction layer was measured in micrometer using a standard microscale calibrated FE-SEM.

**EVALUATING THE NANOLEAKAGE**

The root apices of the remaining five teeth of each group were covered with sticky wax (Dentsply DeTrey, Bois Colombes, France), while their entire surfaces were coated with two layers of nail varnish except for the cervical margin of the restored cavity and 1 mm around. A modified silver staining technique (Tay et al., 2002) was used with basic 50wt% ammoniacal silver nitrate (pH=9.5). The chemical reagents from which the staining solution was prepared are listed in table 1. The solution was freshly prepared by dissolving 25 g of silver nitrate crystals in 25 ml of distilled water. Concentrated (28%) ammonium hydroxide was used to titrate the black solution until it became clear as ammonium ions complexed the silver into diamine silver ([Ag(NH₃)₂]⁺) ions. This solution was diluted to 50 ml with distilled water to achieve a 50wt% solution. The teeth were immediately immersed into the freshly-prepared ammoniacal silver nitrate solution in total darkness for 24 h, followed by thorough rinsing with running distilled water for 5 min. The stained teeth were then placed in a photo developing solution for 8 h under fluorescent light to reduce the diamine silver ions into metallic silver grains within the voids along the bonded interfaces. After removal from the developing solution, the teeth were placed under running distilled water for 5 more minutes. The stained teeth were then sectioned and their cut surfaces were finished, polished and acid-treated following the previously mentioned conditions. The measurements of silver penetration were calculated directly on the SEM monitor, using a multi-point measuring device, observing all the internal cavity margin lengths, in steps of approximately 100 µm. The leakage values were expressed as the sum of the cavity margin lengths showing silver deposition. A comparison between these lengths among the three adhesive systems was possible since the dimensions of all cavities were standardized as mentioned before.

**STATISTICAL ANALYSIS**

The data were statistically analyzed using SPSS statistical package version 10. The data were examined for normal distribution using Kolmogorov-Smirnov test. One way analysis of variance ANOVA was used to compare the values of the three groups. The Tukey's post hoc analysis was used to confirm the significance of differences detected between the test groups and the control. The test was considered significant when p<0.05 and highly significant when p<0.001.

**RESULTS**

SEM images representing the adhesive/dentin (A/D) interfaces (Fig 1-3) revealed a variation in the thickness of the detected interaction layers between the tested adhesive systems. ANOVA indicated significant differences between thickness of interaction layers of tested adhesives (p<0.001) (Table 2). These differences were confirmed among the mean values of Ex and GB (Tukey's comparison, p<0.001) as well as among the mean values of Ex and APL (Tukey's comparison, p<0.05).
Penetration of silver along the cavity walls was observed in all samples. (Fig.4-6). Table 3 summarizes the results of nanoleakage testing. ANOVA indicated significant differences between the mean values of the sum of the cavity margin lengths showing silver deposition in the tested adhesive systems (p<0.001). These differences were confirmed among the mean values of GB and APL (Tukey's comparison, p<0.001) as well as among the mean values of Ex and APL (Tukey's comparison, p<0.05).

Table 2: Means and standard deviations of thickness of the interaction layer (µm) in tested adhesive systems

<table>
<thead>
<tr>
<th>Adhesive systems</th>
<th>Mean ± SD</th>
<th>95% confidence intervals</th>
<th>ANOVA p-value</th>
<th>Tukey’s p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>0.75 ± 0.16</td>
<td>(0.49 - 1.01)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>APL</td>
<td>1.65 ± 0.23</td>
<td>(1.36 - 1.94)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ex (Control)</td>
<td>2.22 ± 0.22</td>
<td>(1.93 - 2.52)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Caption:

Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
### DISCUSSION

Fear of bacterial ingress at the restoration/tooth interface and the subsequent cavities recurrence has pushed the interest of researchers to assess the adaptation and adhesion of restorative materials against tooth cavity walls. Several studies\(^2\),\(^16\),\(^17\) had the concern to evaluate the source of adhesive-dentin bond through assessing the interface characterization. In this in vitro study, it is evident that the G-Bond created a thinner interaction zone in comparison to those formed with other adhesives (Figs1-3). Both the Ex and APL formed traditional hybrid layers as a result of resin infiltration within the demineralized dentin through the collagen fibrils. However, the demineralization in case of Ex seems to be more deep as a result of longer time of acid contact (30s). This assumption could explain the formation of the thicker hybrid layer\(^17\). The interface formed by GB looks different as the surface of dentin is decalcified only slightly and there is almost no exposure of collagen fibrils.

This suggest that functional monomers contained in the bonding material react with hydroxyapatite to form insoluble calcium, forming a thin transitional zone that could be measured in nanometers, so it could be termed a nano-interaction zone with the ability to reduce the risk of bond deterioration\(^-18\),\(^19\)

Moreover, evaluating the microleakage and recently the nanoleakage represent important means of assessing the defective adaptation of materials\(^20\). Although the use of silver nitrate dye was effective in detecting the nanoleakage\(^3\),\(^4\), this in vitro study used the ammoniacal silver nitrate dye because of its efficiency doing the same job and to eliminate the drawback of the regular silver nitrate demineralizing the dentin\(^10\),\(^15\).

SEM images (Figures 4-6) indicated the presence of nanoleakage in all samples bonded with different systems as manifested by the infiltration of silver ions at the adhesive/dentine interface and in some instances around the collagen fibrils. These findings could be attributed to the effect of resin contraction at the time of its polymerization\(^21\).

In addition, the improper wetting of the resin to dentin and collagen surfaces could exist as a result of the wet nature of dentin tissues and bonding resin viscosity\(^22\). The previously nominated factors could create nanogaps between the bonding resin and dentin surfaces. Confirming the same assumption, other researchers have related the occurrence of nanoleakage to the presence of areas of imperfect resin infiltration, retained water or other solvent, poor polymerization, or phase separation\(^22\),\(^23\).

On the other hand, various degrees of silver penetration and brightness were evident between samples of different groups indicating different degrees of nanoleakage. This result could be related to the chemical nature of the adhesive systems themselves that is reflected on the expected degree of polymerization shrinkage and the degree of water sorption (dye solution). The recently-marketed adhesive system, G-Bond, includes water and organic solvents in its chemical formula to improve resin infiltration within the wet dentin substrate, as well as nanofiller particles to reduce the extent of polymerization contraction\(^24\),\(^25\).

A study by Pashley EL et al.,\(^26\) stated that when HEMA is included as a polymerizable solvent for resin monomers, residual water may persist during evaporation as a result of lowering the vapor pressure of water by HEMA. In previously published TEM observations\(^22\), pure polymerized HEMA permitted extensive silver impregnation. The authors

### Figure 8

![Figure 8](image)

**Figure 8**

Table 3: Means and standard deviations of the sum of cavity margin lengths (µm) showing silver deposition in tested adhesive systems

<table>
<thead>
<tr>
<th>Adhesive system</th>
<th>Mean ± SD</th>
<th>95% confidence intervals</th>
<th>ANOVA</th>
<th>Tukey's p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>12.70 ± 2.17</td>
<td>8.76 - 16.64</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>APL</td>
<td>15.34 ± 1.85</td>
<td>11.32 - 19.35</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Ex (Control)</td>
<td>14.74 ± 1.03</td>
<td>14.30 - 15.19</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

\(p^a\): Tukey’s comparison among the mean values of GB and APL

\(p^b\): Tukey’s comparison among the mean values of APL and Ex

\(p^c\): Tukey’s comparison among the mean values of GB and Ex

On the other hand, various degrees of silver penetration and brightness were evident between samples of different groups indicating different degrees of nanoleakage. This result could be related to the chemical nature of the adhesive systems themselves that is reflected on the expected degree of polymerization shrinkage and the degree of water sorption (dye solution). The recently-marketed adhesive system, G-Bond, includes water and organic solvents in its chemical formula to improve resin infiltration within the wet dentin substrate, as well as nanofiller particles to reduce the extent of polymerization contraction\(^24\),\(^25\).

A study by Pashley EL et al.,\(^26\) stated that when HEMA is included as a polymerizable solvent for resin monomers, residual water may persist during evaporation as a result of lowering the vapor pressure of water by HEMA. In previously published TEM observations\(^22\), pure polymerized HEMA permitted extensive silver impregnation. The authors
stated that when HEMA is copolymerized with other resin monomers in the presence of water, it could form hydrogel in the hybrid layer, resulting in additional silver filled patterns in the interfacial layer. In the present study, both APL and Ex contain HEMA and water which could be the reason of the higher degree of nanoleakage. However the presence of alcohol solvent in the Ex formulation could improve the resin infiltration and accordingly reduces the degree of nanoleakage in comparison to the solvent-free adhesive APL.27

**CONCLUSION**

Within the limitation of this study, the hypothesis that G-Bond adhesive system can offer better adhesion to cavity walls is acceptable. The formation of a nano-interaction layer seems to play an important role in minimizing the degree of nanoleakage at the bonding interface.

Clinical relevance: Using the G-Bond adhesive system for bonding composite restorations can minimize the expected degree of nanoleakage and accordingly could reduce the possibility of the future caries recurrence.

**References**

25. Product brochure, GC G-Bond, Advanced Seventh Generation Bonding, GC America Inc., USA.
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