Morphological Characteristics of Self-Etch Adhesives Bonding to Non-Carious Cervical Sclerotic Dentin

Yang Yu and Ru Wang

Department of Stomatology, First Affiliated Hospital, Dalian Medical University, Dalian, China
(Accepted for publication, June 27, 2014)

Abstract: The objective of this study was to observe the morphological characteristics of self-etch adhesives bonding to non-carious cervical sclerotic dentin using Confocal Laser Scanning Microscope (CLSM). Thirty human premolars with buccal non-carious cervical sclerotic lesions were selected, and artificially prepared wedge-shaped lesion was made in lingual surface of this tooth. The dentin lesions were bonding with one-step self-etch adhesive (XENO III and G-Bond Plus) and two-step self-etch adhesive (Clearfil SE Bond) according to the manufacturer’s instruction, respectively. The bonding adhesive component was labeled with 0.1 wt.% Rhodamine B Isothiocyanate. The hybrid layer and resin tags of resin-dentin interface were observed using CLSM. Statistical analysis showed that there were significant differences between non-carious sclerotic dentin and normal dentin in the thickness of hybrid layer and length of resin tags (p < 0.05). The self-etch adhesives significantly affected the hybrid layer thickness and resin tags length between resin and normal dentin interface (p < 0.05), while for resin-sclerotic dentin interface, no significant difference was observed (p > 0.05). The sclerotic dentin presented thinner hybrid layer and shorter resin tags than the normal dentin. There were similar morphological characteristics of resin-sclerotic dentin interface using one-step and two-step self-etch adhesives.

Key words: Dentin bonding, Sclerotic dentin, Confocal Laser Scanning Microscope, Self-etch adhesive, Dentin infiltration

Introduction

Non-carious cervical sclerotic dentin lesions were characterized by a slow and gradual loss of tooth substances resulting in smooth, wedge-shaped defects along the cement-enamel junction. The dental hard tissue loss is a very common clinical condition given that the prevalence and severity of these lesions has been found to increase with age. Accordingly, cervical sclerotic dentin was a clinically relevant bonding substrate in which the dentin has been physiologically and pathologically altered. The dentin surface was covered with hypermineralized layer due to partial or complete obliteration of the dentin tubules with tube- or rod-like sclerotic casts. Therefore, non-carious cervical sclerotic dentin was found to be more difficult to adhere to than normal dentin in the absence of undercut retention.

The bonding efficiency to dentin is believed to depend mostly on micromechanical retention promoted by resin infiltration into demineralized dentin with consequent hybrid layer and resin tags formation. According to the bonding strategies, nowadays available adhesives are classified as etch and rinse system and self-etching system. The etch and rinse system requires phosphoric acid etching and rinsing steps prior to applying adhesive, which promotes the most complete removal of the smear layer. Whereas, Self-etch adhesives simultaneously demineralize and penetrate into the smear layer and underlying dentin collagen, with no rinsing. The self-etch adhesives have gained widespread application in dental clinic due to simplifying the bonding procedures, reducing clinical application time and decreasing technique sensitivity. Self-etch adhesives differ from each other in many respects, such as the number of application steps, acidic monomer composition, water content and acidity. They can be classified as one-step or two-step depending on whether a self-etching primer and adhesive resin are separately provided or are combined into one single solution.

It has been proved that characteristics of smear layer could influence the bonding efficacy of self-etch adhesives. However, for the dentin surface of non-carious cervical sclerotic lesion, the obliteration of dentin tubules and the surface hypermineralized layer may create additional diffusion barriers for self-etch adhesives to form a hybrid layer at the resin composite dentin interface. Previous studies have indicated that bond strength of some contemporary adhesives to non-carious cervical sclerotic dentin was lower than those bonded to normal dentin.
addition, micromorphological studies of resin-dentin interface may also provide additional information about the adhesive performance. These studies commonly applied scanning electron microscopy (SEM) or transmission electron microscopy (TEM) to reveal the bonding interfacial structure. However, little has been reported in literatures about the morphological characteristics of self-etch adhesives and non-carious cervical sclerotic dentin interface using a confocal laser scanning microscope (CLSM).

Therefore, the purpose of this study was to observe and compare the morphology of non-carious cervical sclerotic dentin bonding interface generated by three self-etch adhesives using a CLSM.

Materials and Methods

Thirty human premolars with buccal non-carious cervical sclerotic lesions, were stored in 0.5 % chloramine at 4 °C for a maximum of 1 month after extraction in order to avoid microbial contamination. Sclerotic dentin in non-carious cervical lesions were visually identified according to the North Carolina Dentin Sclerosis Scale: category 1 – little translucency or transparency is evident; category 2 – irregular translucency over less than 50 % of the surface area; category 3 – irregular translucency or translucency over 50 % of the surface area; and category 4 – the majority of the dentin exhibiting translucency or transparency.

Non-cervical cervical sclerotic lesions in category 3 or 4 sclerosis degrees were included in this study. The lesions were limited in depth from 1.5 to 2 mm with a periodontal probe. Artificial wedge-shaped lesions (about 2.0×2.0×1.5 mm) were made in lingual cervical region of this tooth by means of a diamond bur (TR-13, Mani, Tokyo Japan), which was mounted in a high-speed handpiece under copious air-water cooling. Thirty teeth with non-carious sclerotic and artificial cervical lesions were randomly divided into three groups according to the self-etch adhesives (n = 10).

Three commercially available self-etch adhesives were used in this study, including one two-step self-etch adhesive (Clearfil SE Bond) and two one-step self-etch adhesives (XENO III and G-Bond Plus) (Table 1). The lesions were restored by the resin composites (Filtek Supreme, 3M ESPE, USA) in bulk and light cured with a halogen lamp (Mini LED, Satelec, France) with an intensity of 1100 mw/cm² for 20 seconds. For the ensuing CLSM examinations, the adhesive had initially been mixed with fluorescent dye (Rhodamine B Isothiocyanate, C₂H₃Na₂C₅N₅O₅S, Sigma, USA; concentration 0.1 wt %). The self-etching primer was mixed with 0.1 wt.% Rhodamine B Isothiocyanate for Clearfil SE Bond system as previously described. The bonded specimens were stored in physiological saline at 37 °C for 24 h. These teeth were sectioned to obtain a thickness of 2.0 mm specimen, longitudinally through the lesion and approximately parallel to the long axis of each tooth’s clinical crown, using a slow-speed diamond saw under copious waster. The specimens were ground with 400, 800, and 1200-grit, wet silicon-carbide abrasive paper. These specimens were storage in physiological saline at 37 °C for CLSM observations.

The measurements of hybrid layer and resin tags were completed on the gingival dentin of lesions using a Leica TCS-SP2 CLSM (Leica, Heidelberg, Germany) equipped with objectives (40×, NA:0.75, Plan2 Apochromat; 10×, NA:0.4). In fluorescent mode, the laser was operated with a 590 nm short-pass filter (excitation light at 543 nm). Histotomographic images (parallel to the surfaces) were recorded within the specimens, approximately 10 µm under the sections. The thickness of hybrid layer and length of resin tags in the bonding interface were measured via Quantify/Prof. software installed on CLSM in fluorescent mode. Five measurements were performed on each specimen and the mean of hybrid layer thickness and resin tag lengths were calculated. One-way ANOVA was used to identify whether there were any statistically significant differences in hybrid layer thickness and resin tag lengths using different self-etch adhesives (SPSS 16.0; SPSS, Chicago, IL, USA). Post-hoc multiple comparisons were made with the Tukey tests (α = 0.05).

The examination was permitted from the Ethical Committee of the First Affiliated Hospital, Dalian Medical University.

Results

The CLSM images of the resin-dentin interfaces of the adhesive systems are shown in Fig. 1. All of the self-etch adhesives formed the hybrid layer and resin tags in both non-carious cervical sclerotic dentin and normal dentin. The dye penetration and the uniform depth of hybrid layers were observed in normal dentin bonding interface for each self-etch adhesive. And no separation between bonding adhesives and dentin surface were found in specimens. The dye penetrated into the dentin tubules and formed the resin tags, which was regularly dense cones using Clearfil SE Bond into normal dentin (Fig. 1a). On the contrary, there were shorter and less resin tags and no uniform hybrid layers in the interface between the non-carious cervical sclerotic dentin and adhesives (Fig. 1b). With regard to Xeno III and G-Bond Plus, similar morphological appearances of resin-dentin interface for bonding to sclerotic and artificial dentin lesions were observed (Fig. 1c to f).

The thickness of hybrid layer and length of resin tags of each self-etch adhesive into the non-carious cervical sclerotic and artificial dentin lesions are shown in Table 2 and Table 3. There were variations in the thickness of hybrid layer and length of resin tags, depending on the bonding system and the dentin substrate. The hybrid layers were thinner and the resin tags were shorter in sclerotic dentin than in normal dentin. Pair-t test showed that there were significant differences between non-carious sclerotic dentin
Table 1. Composition and Application Mode of Self-Etch Adhesives

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearfil SE Bond</td>
<td>Kuraray, Osaka, Japan</td>
<td>Primer: MDP, HEMA, water, hydrophilic DMA, camphoroquinone, N-diethanol-p-toluidine</td>
<td>Primer was applied and rubbed for 20 s and then air burst</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adhesive: Bis-GMA, MDP, HEMA, hydrophobic DMA, camphoroquinone, N-diethanol-p-toluidine, silanized colloidal silica</td>
<td>Adhesive was applied, gently air burst, and light cured for 20 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent: ethanol</td>
<td></td>
</tr>
<tr>
<td>XENO III</td>
<td>Dentsply/DeTrey, Kontanz, Germany</td>
<td>Primer: HEMA, water, ethanol, BHT, stabilizer, nanofiller</td>
<td>1 drop liquid A was mixed with 1 drop liquid B for 5 s, applied and rubbed for 20 s, then gently air burst, and light cured for 20 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adhesive: PEM-F, UDMA, camphoroquinone, EPD Pyro-EMA, BHT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent: ethanol</td>
<td></td>
</tr>
<tr>
<td>G-Bond Plus</td>
<td>GC Tokyo, Japan</td>
<td>Phosphoric acid ester monomer, 4-MET, dimethacrylate monomer, water, acetone, photoinitiator, nano-silica filler</td>
<td>Pack was activated, the adhesive was applied and rubbed for 10s, strong air burst for 5s, and light for 10s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent: acetone</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Thickness of Hybrid Layer in Resin-Dentin Interface for Each Self-Etch Adhesive (in μm).

<table>
<thead>
<tr>
<th></th>
<th>SE Bond</th>
<th>Xeno III</th>
<th>G Bond</th>
<th>p values of One-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial lesions</td>
<td>2.51±0.68&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>3.10±0.75&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.16±0.64&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.018</td>
</tr>
<tr>
<td>Non-carious Sclerotic lesions</td>
<td>1.83±0.30&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.11±0.71&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.74±0.26&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.202</td>
</tr>
<tr>
<td>p values of Pair-t test</td>
<td>0.012</td>
<td>0.019</td>
<td>0.021</td>
<td></td>
</tr>
</tbody>
</table>

Numbers represent the significant differences between self-etch adhesives

Table 3 Length of Resin Tags in Resin-Dentin Interface for Each Self-Etch Adhesive (in μm).

<table>
<thead>
<tr>
<th></th>
<th>SE Bond</th>
<th>Xeno III</th>
<th>G Bond</th>
<th>p values of One-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dentin</td>
<td>17.13±3.38&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>21.54±4.72&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.36±2.92&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.005</td>
</tr>
<tr>
<td>Sclerotic dentin</td>
<td>10.23±2.78&lt;sup&gt;1&lt;/sup&gt;</td>
<td>12.63±3.95&lt;sup&gt;1&lt;/sup&gt;</td>
<td>9.26±3.42&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.094</td>
</tr>
<tr>
<td>p values of Pair-t test</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Numbers represent the significant differences between self-etch adhesives

and normal dentin in the thickness of hybrid layer and length of resin tags (p < 0.05) (Table 2 and Table 3). One-way ANOVA showed that the self-etch adhesives significantly affected the thickness of hybrid layer and length of resin tags between resin and normal dentin interface, while for non-carious sclerotic dentin substrate, there was no significant difference (p > 0.05).

**Discussion**

The mechanism of modern adhesion between dentin bonding system and dentin substrate is currently believed to be the formation of a hybrid layer and resin tags composed of the resin infiltration into demineralized dentin surface, thus producing micromechanical retention<sup>4,17</sup>). Therefore, the hybrid layer by resin penetrated into the exposed dentinal collagen layer appears to be essential for a reliable dentin bonding<sup>18</sup>.

Common methods used to observe the bonding interfacial structure such as hybrid layer and resin tags in dentin are SEM and TEM. SEM shows the surface details of the samples by etching and drying process. TEM requires an embedding process. Such procedures might lead to shrinking or cracking artefacts in the bottom bonding interface and alteration of the bonding structure<sup>19</sup>.

The introduction of CLSM, used in combination with optical microscopy, laser scan and the computer picture procedure, has provided a valuable new technique for the visualization of bonding structures in dentin<sup>20</sup>. CLSM has the advantages of non-destructive imaging, because sample drying is rendered unnecessary, preventing the formation of artefacts. The CLSM method is also proved to have the potential for measuring the thickness of hybrid
Figure 1. CLSM images of the resin-dentin interfaces of the adhesive systems in fluorescent mode: composite resin (C), adhesive (A), dentin (D); Hybrid layer (arrow), resin tags (finger). a and b: Clearfil SE Bond bonding to normal dentin and sclerotic dentin; c and d: Xeno III bonding to normal dentin and sclerotic dentin; e and f: G-Bond Plus bonding to normal dentin and sclerotic dentin.
in adhesives may be able to promote the infiltration of self-etch resin tags. On the other hand, the hydrophilic solvent components does not contain HEMA in the current study may account for short (HEMA) can easily penetrate into dentin.

The small and hydrophilic monomers as hydroxyethyl methacrylate can influence the morphologies of hybrid layer and its thickness to acid demineralization. The small and hydrophilic monomers as hydroxyethyl methacrylate can influence the morphologies of hybrid layer and its thickness to acid demineralization. The small and hydrophilic monomers as hydroxyethyl methacrylate can influence the morphologies of hybrid layer and its thickness to acid demineralization. The small and hydrophilic monomers as hydroxyethyl methacrylate can influence the morphologies of hybrid layer and its thickness to acid demineralization.

In this study, the morphological characteristics of bonded interfaces in CLSM observations showed that self-etch adhesives mixed by the fluorescent dye have infiltrated into both non-carious cervical sclerotic and artificial dentin lesions, in which the hybrid layer and resin tags were formed. The bonded interface of the non-carious cervical sclerotic lesions showed different morphologies from that in artificial lesion prepared in sound dentin. The thickness of hybrid layer was thinner and resin tags were shorter and less in the resin-sclerotic dentin interfaces than in the normal dentin interface using three self-etch adhesives (Fig. 1 b, d and f). In agreement with reported SEM and TEM observations, the thin hybrid layer was formed at the hypermineralized intertubular dentin and resin tags were short or barely developed for the bonded interface of self-etch adhesive bond to non-carious sclerotic. The morphological characteristics of bonding interface were related to the dentin substrate permeability. The presence of the hypermineralized layer within cervical sclerotic dentin would be considered to significantly decrease the dentin permeability compared to the normal dentin, which might cause an unreliable hybrid layer formation.31

The pH value of self-etch adhesives would affect its ability of smear layer dissolution and dentin demineralization. The self-etch adhesives used in this study have different degrees of acidity: Clearfil SE Bond and G-Bond Plus are considered as mild self-etch adhesives with a pH value of 2.0. Xeno III is regarded as a moderate self-etch adhesive with a pH value of 1.4. With respect to the acidity of self-etch adhesives, Xeno III showed longer tags and thicker hybrid layer in the interface between resin and normal dentin, whereas short tags and thin hybrid layer were measured from Clearfil SE Bond and G-Bond Plus. The results of statistical analysis also confirmed that there was significant difference in thickness of hybrid layer and length of resin tags for three self-etch adhesives bonded to artificial lesions (Table 2 and Table 3). However, for non-carious cervical sclerotic lesions, Xeno III created similar hybrid layer thickness and resin tags length with Clearfil SE Bond and G-Bond Plus (p > 0.05). The results showed that the degree of demineralization was similar for sclerotic dentin treated by different self-etch adhesives in this study. The hypermineralized surface of sclerotic dentin was more resistant to acid demineralization.33

The infiltration ability of self-etch adhesives is also dependent on monomers and solvent system in adhesive components, which can influence the morphologies of hybrid layer and its thickness. The small and hydrophilic monomers as hydroxyethyl methacrylate (HEMA) can easily penetrate into dentin. G-Bond Plus which does not contain HEMA in the current study may account for short resin tags. On the other hand, the hydrophilic solvent components in adhesives may be able to promote the infiltration of self-etch adhesives and water in the adhesive may be capable of simultaneously expanding collagen fibrils during the infiltration of solvated comonomers. The solvent of Xeno III includes a combination of water, ethanol and nanofillers. The thickness of hybrid layer and length of resin tags of Xeno III can also be attributed to its components: a hydrophilic solvent (ethanol) and hydrophilic monomer (HEMA) according to the results of this study.

Some investigations indicated that the morphological characteristics of hybrid layer and the tag length were influenced by the dentin position, where the measurement or observation were done. The direction of the tubules directly affects the demineralization of superficial dentin. The perpendicular orientation of the dentinal tubules is associated with longer resin tags. Moreover, the deep dentin is characteristics by larger and more numerous tubules than superficial dentin. Eliguzeloğlu et al. reported the morphological differences were correlated with dentin position of non-carious cervical sclerotic lesion using SEM. They found that the occlusal wall exhibited thinner hybrid layers than the gingival and axial walls. Therefore, in order to determine the effect of different self-etch adhesives on the morphological characteristics of sclerotic dentin, the gingival wall dentin was observed in the current study.

In conclusion, there were similar morphological characteristics of resin-sclerotic dentin interface using one-step and two-step self-etch adhesives.

Reference

7. Tsai YL, Nakajima M, Wang CY, Foxton RM, Lin CP and Tagami J. Influence of etching ability of one-step self-etch adhesives on bonding to sound and non-carious cervical...


27. Perdigao J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater 26: e24-37, 2010