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Original Article

MICROHARDNESS EVALUATIONS OF RESIN-DENTIN BONDING AREAS BY NANO-INDENTATION

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Abstract

The purpose of this experiment was to determine the hardness values of the hybrid layer and its surroundings through the continuous use of a microhardness measuring device. Black’s Class V cavities were prepared in nine dog teeth. The cavities were divided into four groups according to the dentin adhesive system applied. The adhesive systems were: “Bond One System”, “Liner Bond II System”, “One Step System”, and “Single Bond System”. The treated teeth were observed at seven days post-application. Specimens were cross-sectioned perpendicularly or horizontally to the resin-dentin interface and embedded in epoxy resin. Their surfaces were polished. The microhardness of the resin-dentin bonding area was measured with a nano-indentation tester. The hardness values at a point of 10μm distant from the interface in the direction of the dentin differed between systems. It appeared that this was influenced by the presence of the decalcified dentin not impregnated by resin, differences in the chemistry forming the hybrid layer, and the composition of the bonding resin. The hardness of the dentin-bonding interface and its surroundings was determined, and these areas were observed using SEM. Three layers were confirmed: the healthy dentin layer, the composite resin layer, and the hybrid layer, (in which decalcified dentin impregnated by resin and that not impregnated by resin are considered to be mix). In the hybrid layer, no impression was found by SEM although the hardness in the bonding interface was significantly different. These layers appear to be more elastic and softer than the healthy dentin.

Key words: Hybrid layer—Nano-indentation—Adhesive system—Dentin—Composite resin

An overview of this study was presented at the 76th International Association for Dental Research (Jun. 1998, Nice).
INTRODUCTION

The hybrid layer, which has an important role in resin-dentin bonding, is a structure formed by the following process: healthy dentin is decalcified, and then resin infiltrates and diffuses into the decalcified dentin, intertwining with collagen and hydroxyapatite and turning into a hard structure. This structure was first described as an acideric layer insoluble in the hydrochloric acid produced when 4-META/MMA-TBB resin was applied to a dentin surface processed with a solution of 10% citric acid/3% ferric chloride\(^{5}\). Since then, similar results have been confirmed with seven kinds of extremely adhesive resin\(^{2}\). To confirm the existence of the hybrid layer, the composite resin, the hybrid layer, and the healthy dentin have been observed using \(\text{HCl, NaOCl and Argon ion etching}^{1,5,7}\). However there is no published report examining the physical properties of these structures. In this study, we determined the hardness values of the hybrid layer and its surroundings through the continuous use of a microhardness measuring device.

MATERIALS AND METHODS

The subject materials and teeth for this study are shown in Table 1. The bonding systems and the number of experimental teeth are as follows: Bond One System—three teeth; Liner Bond II \(\Sigma\) System—two teeth; One Step System—two teeth; Single Bond System—two teeth. These bonding systems were applied to two healthy adult dogs aged over one year. Three of them, Bond One System, One Step System, and Single Bond System, are wet bonding systems, and the remaining one, Liner Bond II \(\Sigma\) System, is a self-etching primer system.

The methods used in this study are presented in Fig. 1. This study followed the guidelines for the treatment of experimental animals in Tokyo Dental College. Under general anesthesia, a Black’s class V cavity was formed using a carbide bar inserted in the air turbine handpiece with water pouring; in the edge of the cavity, concave pebbles were produced using a diamond instrument; the inside of the cavity was washed with water, dried, washed alternately with 10% hypo-

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<th>Table 1</th>
<th>The adhesive systems used in this study</th>
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<tr>
<td>Adhesive System</td>
<td>Composite Resin</td>
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<tr>
<td>Bond One System</td>
<td>Conquest Crystal</td>
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<tr>
<td>Liner Bond II (\Sigma) System</td>
<td>Clearfil AP-X</td>
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<tr>
<td>One Step System</td>
<td>Aelitefil</td>
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<tr>
<td>Single Bond System</td>
<td>Z-100</td>
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</table>

Fig. 1  Experimental protocol

Fig. 2  Specimen preparation
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chlorite sodium and 3% hydrogen peroxide solution, and washed and dried again. Following the instruction’s for each product, each test material was applied, and the cavity was filled up with the composite resin attached to each product to finish restoration. After seven days observation period, only the crown of each subjected tooth was collected using a diamond disk.

The methods for preparation and observation of the sample are shown in Fig. 2. The details are as follows: the collected crowns were soaked in phosphate buffered formalin for 24 hours; the pulp cavities were filled with autopolymer resin, sealed with manicure, and embedded with epoxy resin; and the samples were cut horizontally or vertically against the bonding interface using a hard tissue cutter. Sections were ground down with 1,200-grit SiC paper and finally with 0.05 μm of alumina to finish. After preparation of the sample, the hardness around the dentin bonding interface was measured with a micro indentation hardness measuring device, ENT-1100 (Elionix, Tokyo). The dentin bonding interface was observed by attaching a CCD camera to the device. The measuring area was set between a point 30μm away from the interface to the direction of the dentin and the dentin bonding interface (Fig. 3). The measuring conditions were as follows: the measuring interval was 10μm in the vertical and horizontal directions against the interface respectively; the test loading was 500 mgf; the loading step was 1.0 mgf; the loading and unloading rate was 0.05 mgf/msec. Under the above conditions, the hardness was measured at 20 continuous points per measuring area. Then, the impression conditions were checked with a 3D roughness analyzer, ERA-8000FE (Elionix, Tokyo), and photographs were taken by SEM. The results were analyzed statistically by Student 𝑡-test.

RESULTS

The following are the results of the hardness measurements taken for each region of the subjects in this study. These values are plotted in Table 2 and Fig. 4. In the Bond One System, the values at the points 30, 20, and 10μm from the bonding interface in the direction of the dentin were 33±19.8, 28.5±12.3, and 15.5±7.2, respectively. The values at the bonding interface and the point

<table>
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<tr>
<th>Bond One System</th>
<th>Liner Bond II System</th>
<th>One Step System</th>
<th>Single Bond System</th>
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<tr>
<td>I 33 ± 19.8&lt;sub&gt;a&lt;/sub&gt;</td>
<td>34.6 ± 23.6&lt;sub&gt;b&lt;/sub&gt;</td>
<td>46 ± 32.1&lt;sub&gt;c&lt;/sub&gt;</td>
<td>32.3 ± 18.4&lt;sub&gt;c&lt;/sub&gt;</td>
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<tr>
<td>II 28.5 ± 12.3&lt;sub&gt;a&lt;/sub&gt;</td>
<td>37.5 ± 18.4&lt;sub&gt;b&lt;/sub&gt;</td>
<td>36.4 ± 25.7&lt;sub&gt;c&lt;/sub&gt;</td>
<td>30.5 ± 15.4&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>III 15.5 ± 7.2&lt;sub&gt;a&lt;/sub&gt;</td>
<td>48.4 ± 46.2&lt;sub&gt;b&lt;/sub&gt;</td>
<td>19.1 ± 8.3&lt;sub&gt;b&lt;/sub&gt;</td>
<td>31.5 ± 16.9&lt;sub&gt;b&lt;/sub&gt;</td>
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<tr>
<td>IV 21.7 ± 17.9&lt;sub&gt;a&lt;/sub&gt;</td>
<td>49.7 ± 36.2&lt;sub&gt;b&lt;/sub&gt;</td>
<td>11.7 ± 7.2&lt;sub&gt;b&lt;/sub&gt;</td>
<td>22.9 ± 10.8&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>V 33.1 ± 12.3&lt;sub&gt;a&lt;/sub&gt;</td>
<td>86.7 ± 73.5&lt;sub&gt;c&lt;/sub&gt;</td>
<td>36.9 ± 13.3&lt;sub&gt;c&lt;/sub&gt;</td>
<td>86.8 ± 55.8&lt;sub&gt;b&lt;/sub&gt;</td>
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Groups with the same superscript letter are not statistically different when analyzed by Student 𝑡-test at 95% confidence level.

I: The values at the points 30μm from the bonding interface in the direction of the dentin.
II: The values at the points 20μm from the bonding interface in the direction of the dentin.
III: The values at the points 10μm from the bonding interface in the direction of the dentin.
IV: The values at the bonding interface.
V: The values at the points 10μm from the bonding interface in the direction of the composite resin.
10μm from the bonding interface in the direction of the composite resin were 21.7±17.9 and 33.1±12.3, respectively. In the Liner Bond II Σ System, the values at 30, 20, and 10μm from the bonding interface in the direction of the dentin were 34.6±23.6, 37.5±18.4, and 48.4±46.2, respectively. The values at the bonding interface and at 10μm from the bonding interface in the direction of the composite resin were 49.7±36.2 and 86.7±73.5, respectively. In the One Step System, the values at 30, 20, and 10μm from the bonding interface in the direction of the dentin were 46±32.1, 36.4±25.7, and 19.1±8.3, respectively. The values at the bonding interface and at 10μm from the bonding interface in the direction of the composite resin were 11.7±7.2 and 36.9±13.3, respectively. In the Single Bond System, the values at 30, 20, and 10μm from the bonding interface in the direction of the dentin were 32.3±18.4, 30.5±15.4, and 31.5±16.9, respectively. The values at the bonding interface and at 10μm from the bonding interface in the direction of the composite resin were 22.9±10.8 and 86.8±55.8, respectively. The hardness values at the point 10μm from the interface in the direction of the dentin in the three wet bonding systems were lower than those of the healthy dentin (at the points 20 and 30μm away from the interface in the direction of the dentin) (p<0.05). In contrast, in the self-etching primer system, the values were similar to those in the healthy dentin (p>0.05).

As a representative examination, the results of the maximum variation at each point in the Single Bond System (sample #12) are shown in Fig. 5. SEM photographs of the impression condition are also shown in Fig. 6. The tool marks from the hardness measuring device can be observed. The positions of the marks in Fig. 6 correspond to those in Fig. 3. Three layers were confirmed; the healthy dentin layer, the composite resin layer, and the hybrid layer (in which decalcified dentin impregnated by resin and that not impregnated by resin are considered to be mixed). In the hybrid layer, no impression was found in the SEM observation (Fig. 6), although...
① The values at the points 10μm from the bonding interface in the direction of the composit resin

② The values at the bonding interface

③ The values at the points 10μm from the bonding interface in the direction of the dentin

④ The values at the points 20μm from the bonding interface in the direction of the dentin

⑤ The values at the points 30μm from the bonding interface in the direction of the dentin

Fig. 5 Variation at each point ("Single Bond System" sample #12)
actual variation could be measured in this layer (Fig. 5 top column).

**DISCUSSION**

The hybrid layer decalcifies the smear layer attached to the surface of the polished dentin under moderate conditions and prevents the degeneration and constriction of the collagen generated secondarily in the decalcified dentin. At the same time, the surface of the dentin is filled with a great deal of monomer, which is likely to be absorbed into the dentin, and a hybrid layer is formed by polymerization of this monomer. This hybrid layer is considered to represent a continuous change in the structure from the bonding interface to the healthy dentin due to the mechanism of its formation. Therefore, there should be little risk of concentration of stress on the bonding interface and little possibility of microlinkage, with resultant protection of the dental pulp\(^6\). The dentin is further decalcified by processing with a highly permeable acid, and the monomer cannot diffuse and impregnate into all of the layer, resulting in the formation of decalcified dentin that has not been impregnated by resin between the hybrid layer and undecalcified dentin. It is considered that this interface is physically weak in bonding\(^3,4\) and clinically provides only minor protection against secondary caries. In consideration of the above, we examined the hardness of the hybrid layer and its surroundings through the continuous use of a microhardness measuring device.

The microhardness measuring device used in this study does not need surface processing such as ion coating and can control a load within the extremely light range from several decades to hundreds of mgf. The measuring interval can be set at several μm by computer control; therefore, measurements can be made for a continuous layer such as the dentin bonding interface area. Because the conditions of the hybrid layer may differ among bonding systems, we examined four systems: Bond One System, One Step System, Single Bond System, and Liner Bond II System. The first three systems are wet ones, in which the decalcified dentin is maintained moist in order to avoid collagen shrinkage and to allow the monomers to diffuse and impregnate. The last is a self-etching primer system. According to Sugisaki’s report\(^8\), in
processing dentin with phosphoric acid, the width of the decalcified layer was approximately 10 to 15 μm and the width of the hybrid layer formed by this process was about 5 μm. When using a primer, washed and dried collagen fibers can repair gaps, and the bonding material is apt to be impregnated. As a result, the hybrid layer formed is almost as wide as the decalcified layer. If so, the results of the present study that the hardness values at the point 10 μm from the interface in the direction of the dentin in the wet bonding systems differed from the value in the self-etching primer system can be considered as follows. In the wet bonding systems using phosphoric acid, the area 10 μm from the interface in the direction of the dentin might be within the decalcified layer not impregnated by resin, resulting in the lower hardness. On the other hand, in the self-etching primer system using a primer, this area might be within the healthy dentin, so the value would be similar to that of the healthy dentin. In the area that was considered to represent the hybrid layer (the bonding interface), the hardness differed between the Wet Bonding System and the self-etching primer system (p < 0.05). It appeared that the design for making the hybrid layer differed between bonding systems and that those conditions and the composition of resin influenced the hardness. However, the formation of the hybrid layer by these bonding systems did not directly improve the mechanical strength over that of the bonding systems used in the past. Further examination is required to determine how the hybrid layer influences adhesion of dental materials.

In this study, no impressions of tool marks were found in the hybrid layer. This layer may be more elastic and softer than the healthy dentin. However, we could not discriminate the area of decalcified dentin impregnated by resin from the area not impregnated by resin in the hybrid layers. Examination of the hardness of this layer in detail at narrower intervals by setting a smaller load will give us better insight into the impregnation of resin into decalcified dentin area.

**CONCLUSION**

To determine the hardness of the hybrid layer and its surroundings, we continuously used a microhardness measuring device. The hardness values at the point 10 μm away from the interface in the direction of the dentin differed between systems. This appeared to be influenced by the presence of the decalcified dentin not impregnated by resin, and by differences in both the chemistry of the hybrid layer and the composition of the bonding resin. The hardness of the dentin-bonding interface and its surroundings was determined, and these areas were observed using SEM. Three layers were confirmed the healthy dentin layer: the composite resin layer, and the hybrid layer, (in which decalcified dentin impregnated by resin and that not impregnated by resin are considered to be mixed). In the hybrid layer, no impression was found by SEM, although the hardness in the bonding interface was significantly different. These layers appear to be more elastic and softer than the healthy dentin.

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