Effects of coating root dentin surfaces with adhesive materials

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The aim of this study was to evaluate the effects of coating root dentin surfaces with adhesives vis-à-vis the prevention of root dentin demineralization. Root dentin surface was ground with #600 SiC, and then either a single coat of Clearfil SE Bond (SE), Clearfil Tri-S Bond (TS), G-Bond (GB), Hybrid Bond (HB-1), or two coats of HB (HB-2) were applied. Specimens were immersed in an artificial demineralizing solution, then sectioned through the center of the root and polished. Thickness of the coating layer and depth of the demineralized dentin layer were measured under a confocal laser scanning microscope (CLSM). Nanohardness values of the coating layer and underlying dentin were measured using a nanoindentation tester. All obtained data were statistically analyzed. Dentin demineralization was not observed in the surface coating groups with the exception of HB-1, and nanohardness of the underlying dentin was comparable to that of normal dentin. Based on the results obtained, it seemed that coating root dentin surfaces with an adhesive material is a promising good practice to prevent demineralization.

Keywords: Self-etching adhesive, Root dentin, Surface-coating

INTRODUCTION

Recent years have seen a rapid increase in the dentate elderly population, especially in developed countries, due to increase in life expectancy, awareness of dental health measures, and availability of dental care delivery systems. A concomitant development is the high incidence of root caries in the middle-aged and elderly, due to gingival recession and exposure of susceptible root surfaces of the retained dentition. Poor oral hygiene and low salivary flow resulting in dry mouths are also potentially important risk factors for root caries.

Several methods have been proposed to prevent root caries initiation and to promote remineralization prior to contemplating invasive treatments. These include the daily use of fluoride-containing mouthrinses or professional application of fluoridated gels and antimicrobial varnishes. However, a simple method to protect the exposed root surfaces from long-term caries attack is not yet currently available. To inhibit the initiation and progression of root caries, especially for elderly patients who require home care, there is indeed an urgent need for simple treatment options to be developed and made easily available.

In this pursuit of a simple preventive treatment option against root caries, the coating of root dentin surfaces with adhesive systems has been investigated. During the last decade, self-etching adhesive systems have become widely used because of a myriad of advantages: simplified bonding procedure that requires only one application step; reduced chairside time; reliable bonding to dentin; and low technique sensitivity and consistent performance that auger well for strong bonding of composite materials to both enamel and dentin.

During cavity preparation for indirect restorations, sealing the exposed dentin with a resin coating has been proposed as a means to protect the tooth preparation. The resin coating is applied to the prepared cavity immediately after tooth preparation and before making an impression by assembling a dentin bonding system and a flowable composite. It has been reported that resin coatings minimized pulp irritation as well as improved the bond strength between resin cement and dentin. Recently, thin-film coating materials based on all-in-one adhesive technology were introduced for resin coating of indirect restorations. The thin coating materials created a barrier-like film layer on the prepared dentin and played an important role in protecting the dentin from physical, chemical, and biological irritation. In addition, these thin-film coating materials reportedly improved the bond strength of resin cements to dentin, thereby preventing marginal leakage beneath inlays or crown restorations. In light of the many benefits provided by such a protective layer, these all-in-one adhesive materials may therefore also have the potential to cover exposed root dentin surfaces and prevent caries formation.

Using several all-in-one adhesive systems, the aim of this study was to examine the effects of coating root dentin surfaces with these materials in relation to preventing root dentin demineralization.
**MATERIALS AND METHODS**

The research protocol of this study was designed according to the guidelines of the Ethics Committee of the Graduate School and Hospital, Tokyo Medical and Dental University.

**Tooth specimens**

A total of 35 intact roots of human premolars, freshly extracted for orthodontic reasons, were used in this study. Prior to extraction, the patients from whom the teeth were extracted gave informed consent to the use of their teeth for this in vitro study. The specimen preparation procedure is illustrated in Fig. 1, where the teeth were stored in distilled water at –20°C until use, and then assigned into seven groups of five teeth each.

The cementum was removed using a carborundum point (HP20, Shofu Inc.) to expose the root dentin surface. The latter was then ground flat with #600 SiC under running water. Roots were obtained by separating them from the crown at the cementoenamel junction with a low-speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under water spray coolant. To prevent root dentin dehydration during the restoration procedure, pulp tissue was left in situ and the cut surface and root apex were sealed with wax (Utility Wax, GC Corp., Tokyo, Japan).

Three grooves (approximately 0.3 mm wide, 3 mm deep) were prepared on the buccal or lingual dentin surface of each root using a low-speed diamond saw (Isomet 1000), whereby the distance between each groove was approximately 5 mm. An MMA-based resin (Super-Bond C&B, Sun Medical, Siga, Japan) was used to fill the grooves without any dentin pretreatment. Surfaces of the filled resin and surrounding root dentin were re-polished flat with #600 SiC, such that this flattened surface level was defined as the original undemineralized level. Following which, the specimen surfaces were coated with two layers of nail varnish with the exception of the ground surface.

**Root dentin surface coating with adhesives**

Four dentin adhesive systems were used in this study: Clearfil SE Bond (SE; Kuraray Medical, Tokyo, Japan), Clearfil Tri-S Bond (TS; Kuraray Medical), G-Bond (GB; GC Corp., Tokyo, Japan), and Hybrid Bond (HB; Sun Medical, Siga, Japan). Clearfil SE Bond was a two-step self-etching adhesive system, while Clearfil Tri-S Bond and G-Bond were all-in-one adhesive systems. Hybrid Bond was also an all-in-one adhesive system, which was developed to be used as a thin coating material for indirect restorations.

Each adhesive system was applied as a coating material on the root dentin surface according to the manufacturers’ instructions (Table 1). A single coat of SE, TS, and GB was applied on the root dentin surface, while HB was applied in a single coat (HB-1) or a double coat (HB-2). A halogen light curing unit (Optilux 501, Demetron-Kerr, Danbury, CT, USA) was used to polymerize the adhesive resins in this study. Root dentin surface without resin coating was used as

![Diagram of specimen preparation procedure](image-url)
Table 1: Materials used in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Material</th>
<th>Manufacturers</th>
<th>Batch no.</th>
<th>Composition</th>
<th>Application instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>Clearfil SE Bond</td>
<td>Kuraray Medical, Tokyo Japan</td>
<td>11391</td>
<td>Primer: MDP, HEMA, PI, acetylators, CA, water Adhesive: MDP, HEMA, Bis-GMA, MFM, PI, acetylators, CA, microfiller</td>
<td>Apply the primer on the surface for 20 s. Air-blow and apply the adhesive. Then, air-blow and light-cure for 10 s.</td>
</tr>
<tr>
<td>TS</td>
<td>Clearfil Tri-S Bond</td>
<td>Kuraray Medical, Tokyo, Japan</td>
<td>011142</td>
<td>MDP, HEMA, Bis-GMA, PI, water, ethanol, microfiller</td>
<td>Apply the adhesive on the surface for 20 s. Air-blow for 5 s and light-cure for 10 s.</td>
</tr>
<tr>
<td>GB</td>
<td>GC G-BOND</td>
<td>GC, Tokyo, Japan</td>
<td>0503071</td>
<td>4-MET, MDP, PI, water, acetone, microfiller</td>
<td>Apply the adhesive on the surface for 10 s. Air-blow for 10 s and light-cure for 10 s.</td>
</tr>
<tr>
<td>HB</td>
<td>Hybrid Bond</td>
<td>Sun Medical, Siga, Japan</td>
<td>LG1 LE1</td>
<td>Cata-brush: p-amino p-sulfonic acid Liquid: 4-META, methacrylate ester, multi-functional acrylate, methacrylate, water, acetone</td>
<td>Apply the adhesive on the surface for 20 s. Air-blow for 10 s and light-cure for 5 s.</td>
</tr>
</tbody>
</table>

Abbreviations: MMA = methyl methacrylate; MDP = 10-methacryloxydecyl dihydrogen phosphate; Bis-GMA = bisphenyl glycidal methacrylate; 4-MET = 4-methacryloxyethyl trimellitic anhydride; HEMA = 2-hydroxyethyl methacrylate; MFM = multifunctional methacrylate; PI = photoinitiator; CA = catalyst.

a control.

After storage in distilled water at 37°C for 1 day, the specimens were divided into their individual groups and subjected to the following demineralization challenge: 100 ml of an acid buffer solution (2.2 mmol/L CaCl₂, 2.2 mmol/L Na₂HPO₄, and 50 mmol/L acetic acid adjusted to pH 4.5 with NaOH) for 3.5 days at 37°C, with the exception of five undemineralized control specimens.

Confocal laser scanning microscopic (CLSM) observation

The specimens were removed from the demineralizing solution and thoroughly rinsed under running water for 15 seconds. A cut parallel to the long axis of the root through the center of the root was made using a water-cooled, low-speed diamond saw, resulting in two halves (Fig. 1). Each sectioned specimen was then embedded in an epoxy resin (Epon 815, Nissin EM Co. Ltd., Tokyo, Japan) for 24 hours. The surfaces of the sectioned specimens were polished consecutively with #600, #800, #1,000, and #1,200 SiC, followed by diamond pastes (6, 3, 1, and 0.25 µm; DP-Paste, Struers A/S, Copenhagen, Denmark). The polished surfaces were cleaned in an ultrasonic bath, and then observed under a CLSM (1LM21, Lasertec, Kanagawa, Japan) at ×200 magnification (×20 objective lens). The undemineralized control specimens were also treated in Fig. 2 CLSM observations of depth of demineralized dentin and thickness of coating layer.

Fig. 3 Indentation locations in the dentin layer to measure the nanohardness of root dentin.
the same manner as described above.

The features evaluated were depth of the demineralized dentin layer beneath the surface coating and the thickness of the resin coating layer (Fig. 2). The depth of demineralized dentin layer was measured from the original undemineralized level — defined by the top surfaces of the two adjacent MMA-based resin blocks at both sides — to the demineralization front. Measurement was performed at three points for each specimen — namely the midpoint of the specimen which was between the two resin blocks, and two locations which were each 5 \( \mu m \) from the midpoint. The depth of demineralization for each specimen was determined by averaging the values obtained at these three measurement points. The thickness of the coating layer for each specimen was determined by averaging the values at the thickest and the thinnest portions of the coating layer in each specimen.

**Nanoindentation test**

After CLSM observation, an indentation test of the specimens was carried out to confirm the changes in hardness of the coating layers and the root dentin beneath the surface coating after demineralization. The specimens were placed on the stage of a nanoindentation tester (ENT-1100, Elionix, Tokyo, Japan), in which was programmed the locations for indentation. The instrument used for this experiment was a depth-sensing, computer-controlled instrument which had a three-sided pyramidal diamond probe with an included angle of 115°. Load on the indenter was 400 mgf.

Figure 3 illustrates the programmed indentation locations in the dentin layer for both demineralized and undemineralized control specimens. Indentation started at the interface between the surface coating and dentin. Six indentations at 1-\( \mu m \) intervals were made immediately under the coating materials. Five indentations at 3-\( \mu m \) intervals were made in the dentin layer starting from a depth of 5 \( \mu m \), and 13 indentations at 5-\( \mu m \) intervals from the depth of 20 \( \mu m \). These measurements were repeated twice for each specimen to yield a total of 48 indentations. This was so because the dentin layer to be assessed comprised two regions amongst three MMA-based resin blocks. However, in the deeper layer from 80 \( \mu m \) to 250 \( \mu m \), indentations were made at 5-\( \mu m \) intervals. After executing the indentations, nanohardness was calculated using the attached computer. After the nanoindentation test, the geometry of the indentation marks produced in the dentin layer was further confirmed using CLSM.

To measure the nanohardness of the surface coating, nanoindentation was performed at the midpoint of the surface coating for SE, TS, GB, and HB-1. For HB-2, indentation was made at 3 \( \mu m \) above the resin-dentin interface in the lower coating layer, which was presumed to be the same indentation location as HB-1.

**Statistical analysis**

Pertaining to the thickness and nanohardness of the coating materials, the data were compared by one-way analysis of variance (ANOVA) and t-test with Bonferroni correction at 95% level of confidence.

To analyze the nanohardness values in the control groups without surface coating, the data were compared using t-test at 95% level of confidence. Regression analysis was also performed for the nanohardness values of the dentin with/without demineralization from the surface to the depth of 250 \( \mu m \).

To analyze the nanohardness values of dentin with/without surface coating, dentin from the surface to the depth of 80 \( \mu m \) was divided into three regions — namely depth ranges of 0–5 \( \mu m \), 5–20 \( \mu m \), and 20–80 \( \mu m \). For each region, the data were compared by two-way analysis of variance (ANOVA) and t-test with Bonferroni correction at 95% level of confidence. Two factors evaluated were “surface coating” and “depth of demineralized dentin”. To avoid an accumulation of errors due to multiple comparisons, the significance level was modified by Bonferroni correction at 95% level of confidence. Statistical tests were performed using a computerized statistical program (SPSS for Windows Ver. 11, SPSS Inc., USA).

**RESULTS**

**CLSM observation**

The depths of the demineralized dentin layer and the thicknesses of the surface coating materials are summarized in Table 2. Typical CLSM images of

<table>
<thead>
<tr>
<th>Surface coating</th>
<th>Depth of demineralized dentin (( \mu m ))</th>
<th>Thickness of coating material (( \mu m ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (none)</td>
<td>215.5 (2.5)</td>
<td>–</td>
</tr>
<tr>
<td>SE</td>
<td>0</td>
<td>24.1 (0.8)</td>
</tr>
<tr>
<td>TS</td>
<td>0</td>
<td>8.2 (0.7)</td>
</tr>
<tr>
<td>GB</td>
<td>0</td>
<td>6.6 (1.1)*</td>
</tr>
<tr>
<td>HB-1</td>
<td>42.7 (1.1)</td>
<td>5.9 (0.4)*</td>
</tr>
<tr>
<td>HB-2</td>
<td>0</td>
<td>12.7 (1.4)</td>
</tr>
</tbody>
</table>

\( n=5; \) mean (SD)

*Same superscript letter means no significant difference (\( p>0.05 \)).
Fig. 4  CLSM images showing the coating layer and the dentin layer underneath (×200). In all the images, the arrows (→) indicate the “thickness” of adhesive materials and demineralized dentin.

a: control (demineralized); thickness of demineralized dentin layer was approximately 215 µm.
b: SE; no demineralized dentin layer was observed.
c: GB; no demineralized dentin layer was observed.
d: TS; no demineralized dentin layer was observed.
e: HG-1; thickness of demineralized dentin layer was approximately 42 µm.
f: HG-2; no demineralized dentin layer was observed.
Dentin with/without surface coating are shown in Fig. 4. Be it with or without surface coating, all the CLSM images showed that no gaps were formed between the surface coating and dentin. Without surface coating, the mean depth of demineralized dentin was 215.5 µm. With the surface coating of SE, TS, GB, or HB-2, no demineralization of the dentin surface was observed, while there was slight demineralization with HB-1.

Regarding the surface coating, the descending order of thickness was SE > HB-2 > TS > GB > HB-1. However, there was no significant difference in thickness between GB and HB-1 (p>0.05).

**Nanohardness**

Nanohardness values of both the intact and demineralized dentin are shown in Fig. 5. For intact dentin, nanohardness gradually increased from the top to the deeper layers of dentin and reached a plateau at depths exceeding 50 µm. With demineralized dentin, nanohardness was significantly lower than that of intact dentin (p<0.05) and gradually increased from the top surface toward the deeper dentin layers at depths exceeding 200 µm (R²=0.9772, p<0.05). These nanohardness results were in agreement with the results obtained from CLSM observation.

Figure 6 shows the nanohardness values of root dentin with/without surface coating after demineralization exposure. Two-way ANOVA revealed that the nanohardness of demineralized dentin was influenced by the factors of “surface coating” and “depth of demineralized dentin” (p<0.05). Further, there was significant interaction between surface coating and depth of demineralized dentin (p<0.05). At all measurement depths, demineralized dentin without any surface coating exhibited the lowest nanohardness among all the groups — except at 5 µm depth by HB-1 (p<0.05). Up to a depth of 60 µm, there were no significant differences in nanohardness between the intact dentin and the dentin coated with SE, TS, GB, or HB-2 (p>0.05). At depths exceeding 60 µm, there

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**Fig. 5** Nanohardness of root dentin across the longitudinal tooth section. Co (D): control with demineralized dentin; Co (ND): control with no demineralized dentin.

**Fig. 6** Nanohardness of root dentin across the longitudinal tooth section with and without surface coating after demineralization exposure.
were no significant differences in nanohardness among all the coated specimens \( (p>0.05) \). Further, among all the surface-coated specimens, the nanohardness of the specimens coated with HB-1 was consistently the lowest \( (p<0.05) \).

Table 3 shows the nanohardness values of the surface coating materials. SE was significantly harder than the other adhesive resin materials \( (p<0.05) \), whereas the lowest hardness value was obtained with HB-1. The hardness of HB-2 was significantly higher than that of HB-1 \( (p<0.05) \).

<table>
<thead>
<tr>
<th>Surface coating</th>
<th>Nanohardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>45.6 (2.5)</td>
</tr>
<tr>
<td>TS</td>
<td>38.8 (2.1)</td>
</tr>
<tr>
<td>GB</td>
<td>41.2 (1.2)</td>
</tr>
<tr>
<td>HB-1</td>
<td>31.2 (1.9)</td>
</tr>
<tr>
<td>HB-2</td>
<td>44.4 (1.3)</td>
</tr>
</tbody>
</table>

\( n=5; \) mean (SD)

Same superscript letter means no significant difference \( (p>0.05) \)

DISCUSSION

Cementum is the first layer to be encountered when the root surface is exposed to the oral environment. However, the cementum formation exists as a superficial layer on the root and is easily peeled off by intensive root planing during the treatment of periodontal diseases or by toothbrushing. Therefore, the peel-off vulnerability of the cementum layer causes the underlying dentin to be prone to exposure, hence increasing the risk of root caries formation. For this reason, the chief focus of this study was on surface coating of root dentin with a view to preventing root dentin caries formation.

It has been reported that dentin bonding systems, by providing a strong physical barrier with the formation of a hybrid layer, are well poised to be an effective preventive option against root caries\(^{21}\). Indeed, studies have demonstrated that dentin bonding systems could prevent secondary caries formation around and under composite restorations\(^{22,24}\). In particular, self-etching primer adhesive systems have been shown in scanning electron microscope (SEM) studies that they were capable of creating an acid-base resistant zone adjacent to the hybrid layer after acid-base challenge\(^{22,24}\). The exact mechanism by which the acid-base resistant zone is formed still remained unclear, but these findings strongly suggested that the dentin adjacent to the adhesive-dentin interface is different from intact dentin and that it has a limited potential to resist an acid attack from secondary caries. Therefore, reinforced dentin which has an ability to prevent primary caries was termed "super dentin"\(^{25}\).

In the present study, four self-etching adhesive systems were employed for root surface coating. Clearfil SE Bond was a two-step self-etching adhesive system and a water- and solvent-free microfilled adhesive. On the contrary, Clearfil Tri-S Bond, G Bond, and Hybrid Bond were one-bottle/all-in-one adhesive systems, which were in essence water- and solvent-based adhesives. As such, manufacturers of the latter adhesives recommended strong air-blowing before curing to remove both water and solvent in the adhesives, resulting in the formation of a thin adhesive layer. Despite the strong air-blowing, residual water and solvent still remained in the adhesive resins. For this reason, cured all-in-one adhesive systems tend to be more permeable than two-step adhesive systems\(^{26,27}\). On permeability, it is noteworthy that self-etching primer adhesive systems contain one or two different monomers in the composition, which demineralize dentin and promote monomer penetration into the underlying dentin. Clearfil SE Bond and Clearfil Tri-S Bond contained MDP\(^{28}\). Hybrid Bond contained 4-META, while G-Bond contained MDP and 4-MET\(^{29}\) (hydrated 4-META). However, the effect of these acidic monomers on the permeability of coating materials after curing remained unclear\(^{25}\).

To investigate tooth demineralization \textit{in vivo} and \textit{in vitro}, several methods have been employed, such as hardness test\(^{30}\), microradiography\(^{30}\), contact microradiography\(^{30}\), energy dispersive spectroscopy\(^{30}\), and electron probe microanalysis\(^{30}\). In the present study, the effect of root surface coating on prevention of dentin demineralization was evaluated using CLSM observation and nanoindentation test. CLSM evaluation can be performed under near-normal environmental conditions, resulting in fewer artifacts in the specimens\(^{30}\). Subsequently, the nanoindentation test was conducted to confirm dentin demineralization as changes in dentin hardness reflect the mineral loss in dentin after demineralization. By means of ultralight loads, the nanoindentation test is a useful method — which has been used in previous studies — to determine the hardness of small areas such as the dentin-enamel junction, inter- and intra-tubular dentin, and the resin-dentin hybrid layer\(^{37}\).

The current \textit{in vitro} study demonstrated that prevention of dentin demineralization using surface coatings was material-dependent. In particular, two factors were identified to strongly influence the prevention of dentin demineralization: composition of the adhesive material and its thickness. With SE, TS, and GB, root dentin demineralization was completely prevented with only one coat of the adhesive materials. With HB, one coat of the adhesive material (HB-1) resulted in slight demineralization, but demineralization was completely prevented when two coats (HB-2) were applied.

HB was developed as a resin coating material to
protect dentin prepared for indirect restorations\textsuperscript{9}. It was reported that coating the prepared dentin surface with HB resin reaped favorable benefits: improved dentin bond strength\textsuperscript{19} and minimized microleakage in crown restorations\textsuperscript{15}. In terms of material composition, the catalytic system of HB was composed of an aromatic amine and p-sulfonic acid salt in the accessory sponge. In terms of results, the current result showed that one coat of HB yielded the lowest hardness among all the adhesives tested; however, hardness increased with two coats of HB. This suggested that the unpolymerized layer of the first adhesive layer might be hardened upon curing the second layer.

In clinical applications, two mandatory prerequisites for coating materials are durability and wear resistance. In a study by Kaneshiro et al. on the prevention of root surface demineralization, an experimental coating system was investigated by being applied as an appropriately thick layer\textsuperscript{38}. In the current study, only a thin layer of the adhesive materials was applied on the root dentin surface for surface coating. Therefore, future studies should be undertaken to investigate if differences in surface coating thickness would result in in-tandem differences in the durability and wear resistance of the coating layer.

Another practical consideration for clinical applications is biofilm adherence on the coating material. In a study by Daneshmehr et al., it was reported that a root surface coating using a fluoride-releasing all-in-one adhesive was more effective than a fluoride-free adhesive system in preventing biofilm adherence on the root dentin surface\textsuperscript{40}. Similarly, Gyo et al. also evaluated the surface response of resin composites incorporated with a fluorne polymer in relation to inhibiting cariogenic biofilm adherence\textsuperscript{40}. In continuance of this effort against biofilm adherence and formation, a newly developed coating material was investigated using an oral simulator and found to possess self-cleaning properties that inhibited biofilm adherence\textsuperscript{40}. In light of these encouraging results and findings, it may be probable that a root surface coating material which embodies these properties would be developed and available in the near future: possesses good durability and wear resistance, and in particular prevents dentin demineralization and root caries formation.

**CONCLUSIONS**

Within the limitation of this \textit{in vitro} study, it was concluded that root dentin surface coating using all-in-one adhesive systems might be effective in preventing root dentin demineralization. However, the ability to inhibit root dentin demineralization was material-dependent.

**ACKNOWLEDGMENTS**

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