Effect of smear layer treatment on dentin bond of self-adhesive cements

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The purpose of this study was to compare the dentin bond strength of three self-adhesive cements with smear layer pretreatments using a calcium-chelating agent (EDTA) and deproteinizing solution (NaOCl) and to evaluate their interfacial characteristics. Smear layer-covered dentin surfaces were pretreated with EDTA for 60 s, NaOCl for 5 and 15 s, or none. Three self-adhesive cements; Clearfil SA luting (Kuraray Medical), Rely X Unicem clicker (3M ESPE) and Breeze (Pentron) were applied to the dentin surfaces. After 24-h water storage, shear bond strengths to dentin were determined. In addition, nanoleakage evaluation at the interface was performed using FE-SEM and EDS. EDTA-pretreatment significantly improved the bond strength of BR (p<0.05) and NaOCl-pretreatment for 15 s significantly improved the bond strength of RX (p<0.05). On the other hand, for SA, both pretreatments significantly decreased bond strength to dentin (p<0.05). Nanoleakage formation was observed in various amounts at the cement-dentin interfaces.

Keywords: Self-adhesive cement, Dentin, Smear layer, Bond strength, Nanoleakage

INTRODUCTION

Recently, self-adhesive cements have been introduced with the aim of simplifying clinically technique-sensitive multi-step procedures. They are applied directly to the smear layer-covered dentin without any pretreatment procedure. Acidic functional monomers are contained in self-adhesive cements, to simultaneously demineralize and infiltrate into the mineral tissue. However, self-adhesive cements are not able to completely dissolve the smear layer, and their interaction with the dentin surface is very superficial with little formation of a hybrid layer and/or resin tags1–4. Their limited ability to dissolve the smear layer could impair the infiltration of functional monomers into the underlying dentin, which would lead to a ‘weak’ link in the mechanism of adhesion to dentin5. Therefore, several studies have proposed that the smear layer should be treated with a pre-conditioner or self-etching adhesive agent in order to enhance the interaction between the cements and dentin4,6–8.

Clinically, EDTA has been used as a smear layer treatment agent. The calcium-chelating action of EDTA results in removal of the smear layer. Absence of a smear layer would facilitate cement infiltration into the underlying dentin substrate. However, EDTA also creates a thinner decalcified layer in the dentin subsurface, causing exposure of the collagen fibrils on the dentin surface. The self-adhesive cements with their heavy filler load and high viscosity may exhibit limited infiltration into the exposed collagen layer1–4.

On the other hand, the dentin smear layers are composed of disorganized collagen debris as well as mineral particles9,10. The disorganized collagen within the smear layer is not denatured, however after acidic exposure, it forms a gelatinous matrix around the mineral10 and hampers monomer infiltrations. Sodium hypochlorite (NaOCl) solution is a non-specific deproteinizing agent that effectively removes organic components from biological materials at room temperature. When NaOCl solution was applied to smear layer-covered dentin, the mineral to matrix ratio at dentin surface increased and the smear layer was thinned due to dissolution of the collagen part11,12. This conditioned smear layer with less organic components may enhance the bonding performance of self-etching adhesives because the disorganized collagen and the gelatinous layer within the smear layer may prevent resin monomer infiltration and prevent a perfect seal at the resin-dentin interface10,13. It was reported that NaOCl pretreatment also strengthened the bond of self-etching adhesive systems to caries-affected dentin covered with organic-rich smear layer14. Deproteinization of the smear layer might be more effective in enhancing the dentin bonding performance of self-adhesive cements than self-etching adhesives because self-adhesive cements are more viscous than self-etching adhesives.

Nanoleakage is an important indicator when evaluating sealing ability at the interface. This phenomenon is thought to occur due to insufficient infiltration of resin monomer into demineralized dentin or extraction of incomplete polymerized monomers in the submicron interfacial spaces. Recently, it has...
been indicated that nanoleakage not only happens in the hybrid layer, but also in hydrophilic adhesive resin layer. Nanoleakage permits water penetration which may accelerate plasticization of the resin and hydrolysis of exposed collagen within the hybrid layer, resulting in degradation of the adhesive interface. Several studies have demonstrated that self-adhesive cements showed no detectable hybrid layer on smear layer covered-dentin at the micrometer level. However, Rely X Unicem of self-adhesive cement was reported to exhibit nanoleakage formation at the adhesive interface. Nanoleakage at the self-adhesive cement-dentin interface is not well understood.

The adhesion performances of self-adhesive cements to dentin would be dependent upon the monomer formulation, filler load, polymerization behavior and physical properties. Apart from the hybridization concept, other mechanisms (such as chemical interaction) have been advocated to occur at the cement-dentin interface, because the functional group of methacrylate monomers, such as MDP, 4-MET, can interact with calcium ions in the hydroxyapatite. However, in the case of self-adhesive cements, treatment of the smear layer is a problem that still remains to be solved. The purpose of this study was to compare the dentin bond strength of three self-adhesive cements with smear layer treatments using EDTA and NaOCl, and to evaluate their interfacial characteristics. The null hypothesis tested was that smear layer treatments do not influence the bond strengths and silver uptakes at the self-adhesive cement-dentin interfaces.

MATERIALS AND METHODS

Sixty extracted intact human third molars were collected and stored in 4°C in an isotonic saline solution containing a few crystals of thymol to inhibit bacterial growth for a maximum of 6 months until use. Before beginning the bonding experiments, the teeth were retrieved from the disinfectant solution and were stored in distilled water, with daily changes of the latter for two weeks to remove the disinfectant. Approval to use human teeth was obtained from the Human Research Ethics Committee, Tokyo Medical and Dental University, Japan.

Shear bond strength test

The teeth were sectioned mesio-distally at the central groove and the roots removed using a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Sectioned teeth were embedded in a self-curing epoxy resin (Epoxicure, Buehler Ltd, Lake Bluff, IL, USA) in cylindrical rings, with the buccal or lingual surface facing upward and polymerized for 8-h at room temperature. The surfaces were further ground with #600-grit silicon carbide paper under running water for 30 s to create a standardized smear layer and flat dentin surface.

Three self-adhesive cements, Clearfil SA luting (SA) (Kuraray Noritake Dental Inc.; Tokyo, Japan), Rely X Unicem clicker (RX) (3M ESPE; St Paul, MN, USA) and Breeze (BR) (Pentron Clinical Technologies, LLC, CT, USA) were used in this study. The chemical compositions of all the self-adhesive cements used in the experiment are shown in Table 1. All the specimens were randomly divided into four groups (three pretreatment groups and a no-pretreatment group, control), and their dentin surfaces were rinsed with running water for 30 s. For the pretreatment groups, the dentin surfaces were treated with EDTA solution (E-Lize conditioner, Pentron Japan Inc., Tokyo, Japan) for 60 s (EDTA 60 s group), 6% NaOCl (Jiaen 6%, Yoshida Co., Tokyo, Japan) for 60 s (EDTA 60 s group), 6% NaOCl (Jiaen 6%, Yoshida Co., Tokyo, Japan, pH 12.2) for 5 s (6% NaOCl 5 s group) or 15 s (6% NaOCl 15 s group). After pretreatment, the dentin surfaces were rinsed with water from a triple syringe for 30 s and then air-dried for 15 s. The three self-adhesive cements were mixed for 10 s, then applied on a 3-mm-diameter stainless steel rod (SUS-304), which had been sandblasted using 50 μm alumina, and cemented onto the dentin surface by finger pressure. The cement was initially light-cured for 10 s (Optilux 501, Kerr; Middleton, WI, USA) to allow removal of cement excess surrounding the stainless steel rod. Light-curing was performed at the lateral surfaces of the cemented specimens (20 s from different two directions, totaling 40 s of light activation). A load of 500 g was used over the stainless steel rod to standardize the pressure during cementation. After storage in water at 37°C for 24-h, the cemented specimens were mounted in a custom jig and loaded in shear stress at a constant crosshead speed of 1.0 mm/min until fracture by a universal testing machine (Autograph AGS-J, Shimadzu, Kyoto, Japan). After testing, fractured specimens were observed using a stereoscopic zoom microscope (Nikon SMZ1000, Nikon Corp., Kanagawa, Japan) at 120× magnification for failure mode determination. Failure modes were classified as cohesive (within the cement or dentin), adhesive between cement/dentin, or mixed (adhesive and cohesive failure occurred).

The shear bond strength data were analyzed using a two-way ANOVA and Turkey’s multiple comparison at the 95% level of confidence by using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Nanoleakage test by FE-SEM and EDS analyses

In order to observe nanoleakage formation at the cement-dentin interface, three cemented teeth of each group were prepared as previously mentioned for the shear bond strength test with composite blocks instead of stainless steel rods. After storage in water at 37°C for 24-h, the cemented specimens were vertically sectioned using a low-speed diamond saw under water lubrication, across the resin cement/dentin interface, into approximately 1-mm-thick slabs. Two central slabs were chosen from each tooth, forming a total of six specimens per group. Cemented slabs were in turn ground and polished using wet silicone-carbide paper and diamond pastes down to a particle size of 1 μm, then coated with two layers of fast-drying nail varnish applied to within 1 mm of the cemented interfaces. The specimens were immersed in an ammoniacal silver nitrate solution for 24-h, prepared according to the protocol previously described by Tay et
After 24-h in total darkness, the slabs were rinsed thoroughly in distilled water and immersed in a photo developing solution for 8-h under a fluorescent light to reduce silver ions into metallic silver grains. The silver-stained cemented specimens were gently polished with diamond pastes down to a particle size of 1 µm (DP-Paste, Struers, Ballerup, Denmark) and sonicated for 5 min to remove the superficial silver adsorption, and were dried for 24 h at room temperature. After gold-sputter coating, the cement-dentin interface was observed using an FE-SEM (S-4500, Hitachi; Hitachinaka, Japan) at 3,500×, and line scanning analyses for elements including silver, calcium and silicon were performed across the cement/dentin interface by EDS (EMAX-7000, Horiba; Kyoto, Japan).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>SA</th>
<th>RX</th>
<th>BR</th>
</tr>
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<tbody>
<tr>
<td>No treatment</td>
<td>18.8 (1.5)&lt;A&gt;</td>
<td>8.7 (2.4)&lt;B&gt;</td>
<td>13.0 (1.5)&lt;C&gt;</td>
</tr>
<tr>
<td>EDTA 60 s</td>
<td>5.9 (1.3)&lt;B&gt;A&gt;</td>
<td>8.6 (3.3)&lt;A&gt;B&gt;</td>
<td>17.1 (2.4)&lt;B&gt;B&gt;</td>
</tr>
<tr>
<td>6% NaOCl 5 s</td>
<td>12.5 (3.8)&lt;A&gt;C&gt;</td>
<td>8.9 (4.3)&lt;A&gt;A&gt;</td>
<td>8.8 (2.2)&lt;A&gt;C&gt;</td>
</tr>
<tr>
<td>6% NaOCl 15 s</td>
<td>7.9 (2.6)&lt;B&gt;A&gt;</td>
<td>13.6 (2.0)&lt;B&gt;B&gt;</td>
<td>7.4 (1.7)&lt;C&gt;A&gt;</td>
</tr>
</tbody>
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Mean SBS and the standard deviation in MPa (n=10). Different superscript letters indicate statistically differences (p<0.05). Lower case letters refer to column, and upper case letters refer to row.

RESULTS
The bond strength data of the experimental groups are summarized in Table 2. Bond strength was influenced by the material (p=0.019) and by pretreatment (p<0.001). There was a significant interaction between the two independent variables (p<0.001). In the no-pretreatment control group, SA had significantly higher bond strength to dentin than RX and BR (p<0.05). Both EDTA and NaOCl pretreatments significantly reduced the bond strengths of SA (p<0.05). On the other hand, for RX, pretreatment with EDTA did not affect bond strength to dentin (p>0.05), while NaOCl pretreatment for 15 s significantly increased the bond strength (p<0.05), and for BR, EDTA pretreatment significantly increased bond strength to dentin, while NaOCl pretreatment significantly decreased the bond strength (p<0.05).
the groups, the most frequently observed failure modes were adhesive failure or mixed failure with adhesive and cohesive within the cement.

Representative FE-SEM images and EDS analysis data are shown in Figs. 1–3. For the SA specimen, there was little silver deposit at the interface (Fig. 1a), while the RX and BR specimens showed nanoleakage formation with silver deposits above the interface (Figs. 2a) and 3a). The control group specimens exhibited similar demineralization thickness among three self-adhesive cements. In the EDTA pretreatment group, all materials showed silver uptake in the demineralization zone, in which SA and BR were thicker demineralization zone than RX (Figs. 1b, 2b and 3b). In the NaOCl-pretreatment group, SA and BR showed nanoleakage formation with silver deposition above the interface (Figs. 1c and 3c), while for RX specimen, there was little silver deposit at the interface (Fig. 2c).

**DISCUSSION**

The null hypothesis must be rejected because smear layer pretreatments influenced the bond strength and silver uptakes between the self-adhesive cement-dentin interfaces. Their bond strengths to dentin varied according to the smear layer pretreatment with EDTA and NaOCl solutions, depending upon the used cements, and also the silver uptakes, which represent nanoleakage formation, were variously observed at the cement-dentin interface.

For RX, nanoleakage formation with silver deposition was observed at the adhesive interface on no-pretreated dentin, and also on EDTA-treated dentin. In addition, pretreatment with EDTA did not affect bond strength of RX to dentin, which is in agreement with previous studies4,8). In the case of EDTA-treatment to smear layer-covered dentin, the smear layer is removed by the calcium-chelating ability and the underlying dentin also is slightly decalcified, which results in exposure of hydrated collagen fibril layer on dentin subsurface.

**Fig. 1** a) FE-SEM image and EDS result of SA in the no-treatment control group. Metallic silver particles could not be observed at the interface. The elemental energy spectra of Ca increased with a steep slope at the interface. The line analysis was performed along the red line.

b) FE-SEM image and EDS result of SA in the EDTA group. Metallic silver layer could be observed. The elemental energy spectra of Ca increased in a gentle curve and a distinct Ag peak was observed in the demineralization zone. The line analysis was performed along the red line.

c) FE-SEM image and EDS result of SA in the NaOCl-15 s group. Many metallic silver particles could be observed above the interface. The line analysis was performed along the red line. C=resin cement; D=Dentin.
Fig. 2  a) FE-SEM image and EDS result of RX in the no-treatment control group. Some metallic silver particles could be observed at the interface. The elemental energy spectra of Ca increased with a steep slope and a distinct Ag peak was observed at the interface. The line analysis was performed along the red line. b) FE-SEM image and EDS result of RX in the EDTA group. A metallic silver layer could be observed. The elemental energy spectra of Ca increased in a steep slope, which zone is thinner than those of SA and BR (see Figs. 1b and 3b), and a distinct Ag peak was observed in the demineralization zone. The line analysis was performed along the red line. c) FE-SEM image and EDS result of RX in the NaOCl-15 s group. Metallic silver particles could not be observed at the interface. The line analysis was performed along the red line. C=resin cement; D=Dentin.

However, Mazzitelli et al. demonstrated that RX showed no signs of demineralization/infiltration to EDTA-treated dentin. The acidic methacrylated monomer in RX of the solvent-free HEMA-free material has two phosphate groups and a minimum of two C=C double bond units per molecule, which is relatively high molecular weight compared with acidic monomers of MDP in SA and 4-MET in BR. Resin monomer infiltration is proportional to the applied concentration, viscosity of the material, molecular weight or size, the affinity of monomers for substrate and the time allowed for penetration. In this study, the demineralization zones of RX in the EDTA-pretreatment group were thinner than those of BR and SA although the dentin surfaces were similarly pre-treated with EDTA solution. These results would indicate that in RX, exposed collagen fibrils collapsed due to less monomer infiltration. The RX cement might have lower affinity to the EDTA-treated dentin surface covered with hydrated collagen fibrils than BR and SA cements. Additionally, the similar bond strengths of RX between the no-pretreatment and EDTA-treated group might indicate that there is little difference in the affinity to smear layer-covered dentin from the EDTA-treated dentin, because RX cement could partially dissolve the minerals binded in the smear layer, but the disorganized collagen debris of smear layer would be left on the surface.

For the 4-MET/HEMA-containing material, BR, EDTA pretreatment significantly increased bond strength to dentin, and a layer of silver uptake was observed in the demineralized zone, which was thicker than RX. HEMA can promote resin infiltration into hydrated demineralized dentin, which prevents collapse of demineralized zone. This thicker demineralized zone in the case of BR might indicate hybridization of 4-MET/HEMA to EDTA-treated dentin. Hybridization of BR cement would lead to improvement of the bond strength to EDTA-treated dentin. However, water present in the demineralized zone would cause a discrepancy between monomer infiltration and demineralization depths.
and/or poor polymerization, leading to nanoleakage formation in the demineralized zone (Fig. 3b). On the other hand, in the no-pretreatment group (control group), RX represented nanoleakage formation above the adhesive interface (Fig. 3a). According to EDS analysis with line scan of Ca, all materials created only a thin demineralization zone to smear layer-covered dentin, but could not distinguish between their demineralization potentials. However, Lin et al.\textsuperscript{21} reported on the SEM observations of the etching patterns on the enamel surface that SA defined the etched enamel area compared with RX and whereas etched enamel was less defined with BR. The inferior etching potential of BR could not provide optimal infiltration spaces of HEMA to smear layer-covered dentin, and poly-HEMA hydrogels formed with residual water on the adhesive interface permitted water movement, which would produce nanoleakage spaces.

In the case of the HEMA-free material SA, EDTA pretreatment significantly decreased bond strength, and the thicker demineralized zone with silver deposition was observed (Fig. 1b). The creation of a thicker demineralized zone would indicate that the hybridization of SA cement to EDTA-treated dentin was similar to the case of BR. SA uses the phosphoric methacrylated monomer, MDP with lower molecular weight. This functional monomer might have the potential to infiltrate into hydrated collagen fibrils. However, the significant reduction in bond strength and the occurrence of nanoleakage pathways in EDTA-treated dentin would indicate that the hybridization of SA could hardly contribute to performance of bond strength and sealing ability. On the other hand, for the no-treatment group, SA had significantly higher bond strength than RX and BR, in which there was little silver deposition at the interface (Fig. 1a). It is thought that the three materials used in this study have various hydrophobic/hydrophilic properties and etching potentials. SA and RX have higher etching potentials than BR\textsuperscript{21}. The characteristics of EDTA-treated dentin would indicate

![Figure 3](image_url)

**Fig. 3**  
(a) FE-SEM image and EDS result of BR in the no-treatment control group. Many metallic silver particles could be observed at the interface. The elemental energy spectra of Ca increased with steep slope and a distinct Ag peak was observed at the interface. The line analysis was performed along the red line.  
(b) FE-SEM image and EDS result of BR in the EDTA group. Metallic silver layer could be observed. The elemental energy spectra of Ca increased in a gentle curve and a distinct Ag peak was observed in the demineralization zone. The line analysis was performed along the red line.  
(c) FE-SEM image and EDS result of BR in the NaOCl-15 s group. Many metallic silver particles could be observed above the interface. The line analysis was performed along the red line.  
C=resin cement; D=Dentin.
that RX has higher hydrophobicity than SA and BR, and SA of HEMA-free cement would be more hydrophobic than BR of HEMA-containing cement. Proper etching ability would provide cement infiltration spaces due to mineral dissolution from the smear layer and optimum affinity to residual hydrated collagen debris on the smear layer would enhance cement infiltration into the dentin substrate, which might lead to superior bonding efficacy of self-adhesive cements to smear layer-covered dentin.

There have been many studies on the pretreatment effect of NaOCl solution on bonding to smear layer-covered dentin. Several previous studies have demonstrated that NaOCl pretreatment on smear layer-covered dentin had a negative effect on the bond strength of etch & rinse adhesive systems and self-etching adhesive systems. It has been speculated that remnants of super-oxide radicals generated by NaOCl within the dentin surface inhibit polymerization of resin monomers, resulting in significant reduction of bond strength. This negative effect is dependent upon the application time of NaOCl. It has been demonstrated that a 15 s or less application of NaOCl did not significantly affect the bond strength of self-etching adhesive systems to dentin although pretreatment with NaOCl for 30 s or longer compromised the bonding of adhesive systems to dentin. In this study, the NaOCl treatment time was for 5 or 15 s, however unfortunately, the bond strengths of SA and BR significantly decreased. Although there were no remarkable difference in failure modes between the control and NaOCl group in SA and BR, NaOCl pre-treatment produced nanoleakage formations above the dentin surface, not in the dentin substrate. The reduction in bond strength and formation of nanoleakage in the case of the cements SA and BR might be due to poor polymerization induced on the NaOCl-treated dentin. Some researchers have demonstrated a reversal effect of reducing/antioxidant solutions on the compromised bonding of etch & rinse and self-etching adhesive systems to NaOCl-treated dentin. Further research is required to determine the effect of reducing/antioxidant solutions on the compromised bonding of self-adhesive cement to NaOCl-treated dentin.

On the other hand, for RX, the NaOCl pretreatment for 15 s significantly increased bond strength without nanoleakage formation. Some researchers have demonstrated that NaOCl pretreatment was capable of partially removing the organic components and thinning the smear layer. When eliminating organic components from the smear layer, exposed mineral components would increase and water content would decrease. RX cement contains multifunctional methacrylated phosphoric acid ester monomers that are claimed to react with hydroxyapatite. An increase in the mineral content and a reduction in water content on the surface might be advantageous for the hydrophobic RX cement to chemically interact with hydroxyapatite and improve the surface wettability, leading to the establishment of adhesion to dentin. Additionally, the methacrylated phosphoric acid ester monomer is claimed to interact chemically with the basic inorganic fillers in the material, leading to an additional acid-base setting reaction, apart from the free radical polymerization. The setting reaction of RX might be able to resolve the negative effect of polymerization on NaOCl-treated dentin surface.

Within the limitations of this study, it was concluded that smear layer treatment using the calcium-chelate (EDTA) or deproteinating agent (NaOCl) affected the bond strength and sealing ability between the tested self-adhesive cements and the dentin interface, which may be dependent upon the hydrophobic/hydrophilic properties, etching potential and polymerization behavior of the materials. The more hydrophobic the cement, the greater the reduction in surface wetting to hydrated dentin and/or greater induction of hydro-blisters in the cement, leading to nanoleakage formation above the interface. On the other hand, more hydrophilic cements would exhibit better surface wetting of hydrated dentin, however this could compromise long-term stability at the interface due to hydrolytic degradation. Pre-application with a deproteinizing agent could reduce water on the dentin surface due to removal of hydrated collagen debris in the smear layer, leading to improvement in the surface wetting of hydrophobic self-adhesive cement on smear layer-covered dentin. In addition, the potential of acidic functional monomers to chemically interact with hydroxyapatite is important, which would be dependent upon the type of functional monomers with phosphate or carboxylic group in self-adhesive cements, however little is known about them.

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REFERENCES