Evaluation of EDTA Conditioners of Various pHs on Dentin Surfaces

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Abstract

Purpose: The purpose of this study was to evaluate the effect of dentin conditioning by various ethylenediaminetetraacetic acid (EDTA) conditioners of various pHs on dentin surfaces.

Methods: EDTA conditioners at 0.5 mol/l with pH 8.0, pH 10.0, and pH 12.0 were used to remove the dentin smear layer on the surface of extracted human molar dentin. E-Lize Conditioner with pH 7.4 was used as a control. The change in the micro Vickers hardness of the dentin caused by conditioning was measured with a universal testing machine. The wall-to-wall polymerization contraction gap of the resin composite was examined with a light microscope. After conditioning, the dentin surface was observed with a scanning electron microscope (SEM, ×5,000).

Results: No significant differences were observed in the micro Vickers hardness of the dentin between the conditioners at all pHs. In the contraction gap measurements, complete marginal adaptation was observed for the pH 7.4 and 8.0 EDTA conditioners. The SEM observations showed that the removal of the dental smear layer was caused by the change in pH; thus, more of the surface of the dentin smear layer remained as the EDTA pH increased.

Conclusion: The optimum pH for 0.5 mol/l EDTA conditioner is pH 7.4-8.0, which results in complete marginal adaptation.

Key words: dentin conditioning, dentin hardness, smear layer
Introduction

In 1951, Hahn and Reygadas\textsuperscript{1} and Screebny and Nikiforuk\textsuperscript{2} reported the demineralizing effect of ethylenediaminetetraacetic acid (EDTA) on dental hard tissues. Chelators were introduced to endodontics by Nygaard-Ostby\textsuperscript{3}, who recommended the use of a 15% EDTA conditioner (pH 7.3) for removing the smear layer in the root canal dentin wall. EDTA and citric acid chelator solutions are commonly used for root canal treatment\textsuperscript{4}, and are very effective at removing the smear layer. Numerous studies have shown that irrigation with 17% EDTA solution conditions the root canal walls effectively\textsuperscript{5-19}. In 1997, Morgan and Baumgartner\textsuperscript{20} reported that the amount of smear layer removed is related to the pH of the chelating agent and the length of exposure. In 2002, Ahmet and Semra\textsuperscript{21} observed that the amount of phosphorus liberated from dentin increases with EDTA concentration and exposure time, and that the effect is stronger at a neutral pH than at pH 9.0. Usually, 15–17% EDTA is used for root canal treatment. However, it has been reported that root canal irrigation with 15% EDTA results in the removal of the smear layer and the demineralization of the dentin immediately below the smear layer, reducing the hardness. Removal of the smear layer is difficult with low-concentration EDTA solution because EDTA releases hydrogen ions as the chelation reaction progresses, leading to an acidic environment in which chelation is minimized (self-limiting effect). To prevent the self-limiting effect, Nakashima et al\textsuperscript{22} prepared an alkaline EDTA solution by adjusting the pH and then evaluated its ability to chelate calcium acetate and to remove the smear layer. They reported that a 3% EDTA solution (pH 9.0) removes the smear layer without excessive demineralization. The smear layer on the ground dentin surface must be removed\textsuperscript{23-25} prior to the application of bonding agent because the smear layer interferes with bonding between the resin composite and dentin surface. Acid etching of the enamel surface removes the smear layer and the micro-undercut structure, which consists of enamel rods\textsuperscript{26}. However, a dentin conditioner is not always required, although various acid and acidic monomers are used in commercial dentin bonding systems\textsuperscript{27}. Several studies have recommended decalcifying the dentin with a dentin conditioner consisting of citric acid solution containing ferric chloride or phosphoric acid, because the formation of resin tags in the dentin tubules and the formation of a hybrid layer beneath the superficial dentin surface are essential for dentin bonding\textsuperscript{28}.

In 1982, Nakabayashi\textsuperscript{29} reported that bonding between resin and dentin is due to the formation of a hybrid layer in superficial dentin conditioned with citric acid and ferric chloride. However, in 1984, Munksgaard and Asmussen\textsuperscript{30} developed a dentin primer composed of glutaraldehyde and 2-hydroxyethyl methacrylate (2-HEMA), called GLUMA. They suggested that when GLUMA is applied for 60 s to dentin conditioned with EDTA, the aldehyde activates the collagen protein, thereby initiating polymerization with 2-HEMA\textsuperscript{31}.

In 1991, Sugizaki\textsuperscript{32} reported that a dentin primer containing N-methacyrloyl-5-amino salicylic acid expanded the superficial demineralized dentin layer, which had been reduced by etching with citric acid containing calcium chloride. In addition, in 1992 Gwinnett and Kanca\textsuperscript{33} reported that maintaining the acid-etched dentin collagen prevents shrinkage of the collagen network, which improves the blot-dry wet-bonding technique recommended in order to keep the collagen network expanding. Thus, dentin bonding has been discussed in terms of mainly the interaction between the dentin bonding agent and the dentin collagen and the priming effect has been attributed to the physical alteration of the dentin structure through the total-etch wet bonding technique\textsuperscript{34}.

We have previously reported that the dentin conditioner does not decalciﬁ the dentin beneath the smear layer because the contraction gap width of the resin composites in the dentin cavity increases with decalcification and the consequent reduction in the hardness of the conditioned dentin\textsuperscript{35, 36}. To minimize the thickness of the decalcified dentin layer, 0.5 mol/l EDTA is usually used, although the relationship between the application time and the thickness of the softened dentin has not been clarified. In the present study, we evaluated the effect of EDTA conditioners of various pHSs on the dentin surface and the wall-to-wall contraction gap width.

Materials and Methods

This study was conducted with the approval of the
Showa University Institutional Review Board (approval number 2011-016). Sixty-four extracted human molars were used. Experimental dentin conditioners were prepared by adding sodium hydroxide to a solution of EDTA disodium salt (Dojin) until the mixture reached pH 8.0, 10.0, or 12.0. A commercial EDTA conditioner (E-Lize Conditioner; Pentron Japan), pH 7.4, 0.5 mol/l, 14.5%, was used as a control (Table 1).

1. **Measurement of micro Vickers hardness**

A flat dentin surface was prepared on extracted human molars by using wet silicon carbide paper (1,000 grit). The micro Vickers hardness of the dentin was measured using a universal testing machine (HM-103, Mitutoyo) with a load of 15 g for 20 s. The measurements were performed three times and the mean value recorded. The dentin surface was treated with the control 0.5 mol/l EDTA solution at pH 7.4 or an experimental 0.5 mol/l EDTA solution at pH 8.0, 10.0, or 12.0 for 60 s. Five specimens were used for each EDTA solution; thus, 20 specimens in total were prepared.

2. **Measurement of the wall-to-wall polymerization contraction gap**

The proximal enamel of the extracted human teeth was removed using wet silicon carbide paper (600 grit) to create a flat surface, and a cylindrical cavity, approximately 3 mm in diameter and 1.5 mm deep, was prepared in the exposed dentin. The cavity wall was conditioned with an EDTA conditioner for 60 s and the cavity was rinsed and dried. The cavity was then primed with a commercial dentin primer (E-Lize Primer, Pentron Japan) for 60 s and the cavity was dried thoroughly. A commercial dual-cured dentin bonding agent (Clearfil Photo Bond, Kuraray Noritake Dental) was applied to the cavity, excess bonding agent was removed with a gentle air blast, and the cavity was irradiated for 10 s with a commercial halogen lamp unit (G-Light Prima, GC). The cavity was slightly over-filled with a commercial light-activated resin composite (Palifique Estelite Paste A3, Tokuyama Dental) and the composite surface was gently pressed onto a glass plate coated with a polyester matrix. The resin composite was polymerized using a commercial lamp unit for 40 s and the specimens were immersed in water at room temperature for 10 s. The over-filled resin composite was removed with wet silicon carbide paper (1,000 grit) and the exposed resin-dentin margin, including the surrounding dentin surface, was polished with linen cloth and alumina slurry. The marginal adaptation of the resin composite was inspected under an optical microscope and the contraction gap width was measured using a digital microscope mounted on the ocular lens of the microscope. The gap width was measured at eight points at intervals of 45° along the cavity margin and the gap value was calculated the sum of the diametrically opposing gap widths as a percentage of the cavity diameter. The maximum gap value was taken as the gap value for the specimens. Each conditioning group comprised 10 specimens; thus, 40 specimens in total were measured.

3. **Scanning electron microscopic observations**

A flat dentin surface was prepared on an extracted human molar with wet silicon carbide paper (600 grit). The dentin surface was conditioned with one of the EDTA conditioners for 60 s. The specimens were sectioned through the center of the dentin surface, dried with ethanol, critical point dried, vacuum evaporated, and sputter coated with platinum and palladium. The morphological characteristics were observed with a scanning electron microscope (SEM; S-4700, Hitachi).

4. **Statistical analysis**

The micro Vickers hardness and wall-to-wall polymerization contraction gap were analyzed by one-way analysis of variance (ANOVA) and Fisher’s protected least significant difference (PLSD) test. The level of significance was taken as p<0.05.

**Results**

1. **Micro Vickers hardness measurements**

The ratio of the decalcified dentin hardness was calculated by the formula presented in Table 2. No significant difference was found between the tested groups by one-way ANOVA and Fisher’s PLSD test (p<0.05).

2. **Wall-to-wall polymerization contraction gap measurements**

The wall-to-wall contraction gap values for the EDTA conditioners are presented in Table 2. Complete marginal integrity was obtained when the dentin cavity was conditioned with the pH 7.4 and 8.0 EDTA conditioners. A slight contraction gap was observed in the pH 10.0 and 12.0 EDTA conditioner groups, but no significant difference was found between the tested groups by one-way ANOVA and Fisher’s PLSD test (p<0.05).
Table 1  Dentin bonding system tested

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>pH</th>
<th>Molar Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Lize Conditioner</td>
<td>Pentron</td>
<td>110011</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental EDTA</td>
<td></td>
<td></td>
<td>8.0</td>
<td>0.5 mol/l</td>
<td>14.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>pH</th>
<th>Molar Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Lize Primer</td>
<td>Pentron</td>
<td>110011</td>
<td>7.4</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Lot No.</th>
<th>pH</th>
<th>Molar Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonding Agent</td>
<td>Kuraray Noritake Dental</td>
<td>00456B (Catalyst)</td>
<td>00551B (Universal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearfil Photo Bond</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resin Composite</td>
<td>Tokuyama Dental</td>
<td>J321</td>
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</table>

Table 2  Evaluation of EDTA at various pHs

<table>
<thead>
<tr>
<th>EDTA (pH)</th>
<th>Wall-to-Wall Contraction Gap Width (%)</th>
<th>Vickers Hardness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>0 (10)</td>
<td>98.86±13.36</td>
</tr>
<tr>
<td>8.0</td>
<td>0 (10)</td>
<td>97.83±7.32</td>
</tr>
<tr>
<td>10.0</td>
<td>0.026±0.039 (7)</td>
<td>91.82±5.77</td>
</tr>
<tr>
<td>12.0</td>
<td>0.020±0.037 (8)</td>
<td>100.11±10.83</td>
</tr>
</tbody>
</table>

\(^1\)N=10, mean±SD (number of gap-free specimens)
\(^2\)N=5, mean±SD

Data were analyzed by one-way ANOVA and Fisher’s PLSD test (p<0.05)

3. **Scanning electron microscope observations**

The SEM images (Figs. 1~4) showed that efficient removal of the dental smear layer was due to the change in pH value. The smear layer on the ground dentin surface was completely removed by the pH 7.4 and 8.0 EDTA conditioners. The dentinal plugs and the smear layer remained when treated with the pH 10.0 and 120 EDTA conditioners.

**Discussion**

Nakabayashi\(^{30}\) proposed that dentin bonding occurs when a hybrid layer is formed in the superficial dentin substrate. Sugizaki\(^{22}\) and Gwinnett and Kanca\(^{33}\) suggested the priming effect arises from hybrid layer formation because the dentin collagen that collapses during air drying is expanded by the dentin primer\(^{34}\). This explanation of the dentin bonding mechanism conflicts with the fact that the dentin-bonding agent bonds very efficiently to the enamel even though enamel has an extremely low collagen content. We have proposed that the inorganic components rather than the organic components in dentin play an important role in bonding because the contraction gap width increases when the dentin cavity wall is decalcified and softened by dentin conditioner\(^{36}\). In addition, gap formation occurs when the resin monomer is applied without an adhesive monomer. Therefore, the dentin bonding may arise from the interaction between the adhesive monomer in the dentin bonding agent and the inorganic components of the tooth structure. Chiba et al.\(^{36}\) reported that when the dentin substrate is decalcified by phosphoric
Fig. 1 SEM images of dentin surface (left) and cross section (right) after conditioning with pH 7.4 EDTA （×5,000）

Fig. 2 SEM images of dentin surface (left) and cross section (right) after conditioning with pH 8.0 EDTA （×5,000）

Fig. 3 SEM images of dentin surface (left) and cross section (right) after conditioning with pH 10.0 EDTA （×5,000）

Fig. 4 SEM images of dentin surface (left) and cross section (right) after conditioning with pH 12.0 EDTA （×5,000）
acid, a small contraction gap remains even if the tensile bond strength is high. Teduka et al.\textsuperscript{37} reported that complete marginal integrity was achieved when the dentin was conditioned with EDTA for no longer than 8 min and the marginal adaptation was reduced for conditioning times longer than 9 min. They proposed that the critical decalcified dentin thickness for marginal integrity was approximately 1.5 \( \mu \text{m} \). Wu et al.\textsuperscript{35} reported that when the dentin surface was conditioned with 10\% phosphoric acid solution, complete marginal adaptation was not observed for any of the conditioning times from 5 to 60 s. The calcium content after conditioning with 10\% phosphoric acid was reduced even when the conditioning time was only 5 s.

In this study, at higher pH values there was no decalcification and no change in the dentin surface hardness. Although the smear layer remained, a contraction gap was observed when the pH increased above pH 8.0.

Nakashima et al.\textsuperscript{20} reported that in 3\% EDTA the chelating ability was high at high pHs. However, there are factors other than the concentration of EDTA and pH that affect the removal of the smear layer, such as the use of endodontic tools and NaOCl for irrigation\textsuperscript{38,39}. For dentin bonding, the conditioners are applied without any mechanical agitation or auxiliary solutions.

The decalcification of dentin is caused by the decrease in the pH of the EDTA solutions. In this study, the results of micro Vickers hardness testing showed no differences in dentin hardness between pH 7.4 and 8.0, because the concentration of Ca\(^{2+}\) in the dentin might have remained constant under alkaline conditions.

The gaps created by wall-to-wall polymerization contraction suggest that the critical point for dentin bonding is between pH 8.0 and 10.0. This is supported by the fact that Asmussen and Munksgaard\textsuperscript{9,21,40} and Chiba et al.\textsuperscript{36} used a pH 7.4 EDTA solution to achieve high-quality dentin bonding.

The SEM findings revealed that the pH 7.4–12.0 EDTA solutions removed the majority of the smear layer on the intertubular dentin surface. However, the pH 7.4 and 8.0 solutions removed dentinal plugs, which probably produced high-quality bonding between dentin and composite resins\textsuperscript{41}.

**Conclusions**

We conclude that the optimum pH for 0.5 mol/l EDTA conditioner is pH 7.4–8.0, which results in complete marginal adaptation.

**References**

29) Nakabayashi N. Resin reinforced dentin due to infiltration of monomers into the dentin at the adhesive inter-
中性からアルカリ性の EDTA を用いた場合の象牙質スミヤー層除去効果

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抄録
目的：コンポジットレジンの象牙質に対する良好な窩洞適合性を獲得するためには、ボンディング処理に先立ち、窩壁に形成されるスミヤー層を 0.5 mol/l (pH 7.4) EDTA 溶液を用いて完全に除去し、35 vol% Glyceryl mono-methacrylate 水溶液を用いてブライニングを行うことが必要不可欠である。中島らは、アルカリ性の EDTA 溶液を用いることで、self-limiting 効果を軽減、低濃度の EDTA 溶液でもスミヤー層が除去できるこ
to を報告している。そこで今回の研究では、3 種類の異なる pH に調整した試作 EDTA 溶液を用いて歯面処理を行った際のスミヤー層の除去効果を、象牙質硬さの計測および窩洞適合性試験を行うことで評価を行った。

材料と方法：本研究で用いたヒト抜去歯は、昭和大学歯学部歯科保存学講座歯科保存学講座美容歯科学部門の研究に用いられた。酸化ナトリウムを用いて滴定し、pH が 8.0, 10.0, および 12.0 となるように調整を行った。各試作 EDTA 溶液の作用させた後の象牙質表面のピッカース硬さの計測。窩洞適合性試験。電子顕微鏡を用いた微細構造の観察を行った。ピッカース硬さの計測は 20 秒間 15 g の荷重を負荷した。

窩洞適合性試験では、ヒト抜去歯の象牙質に、直径 3.0 mm、深さ 1.5 mm の円柱窩洞を形成し、各 EDTA 溶液で処理後、ブライニングおよびボンディング処理を行い、コンポジットレジンを塗装硬化させ、窩洞に生じるコンタクションギャップの計測を行った。また、日立社製走査型電子顕微鏡 S-4700 を用いて観察を行った。

結果：各 EDTA 溶液で処理を行った際のピッカース硬さの計測値は、すべての試片群で統計学的に有意差が認められなかった。窩洞適合性試験では、pH7.4 と pH8.0 で完全な窩洞適合性が認められた。電子顕微鏡を用いた観察では、pH7.4 と pH8.0 で象牙質表層のスミヤー層が完全に除去されていたのに対し、pH10.0 と pH12.0 では象牙質表層、象牙細管内に残存が確認された。

結論：象牙質に対して良好なコンポジットレジンの接着を獲得するための 0.5 mol/l EDTA の最適 pH は、pH7.4 から 8.0 であることが確認された。

キーワード：象牙質コンディショニング、象牙質硬さ、スミヤー層