Application of Root Canal Cleaning Agents having Dissolving Abilities of Collagen to the Surface Treatment for Enhanced Bonding of Resin to Dentin

Jiro TANAKA and Hiroyuki NAKAI
Department of Dental Materials, Okayama University Dental School, 2-5-1 Shikata-cho, Okayama 700, Japan

Received August 2, 1993/Accepted October 15, 1993

Commercial root canal cleaning agents with the ability to dissolve collagen were used for the surface treatment to enhance the bonding of dental resins. These agents increased the bond strength of a dental bonding agent to dentin. For example, the tensile bond strength to bovine dentin was 10.7±3.9MPa following the joint use of M and N and 8.8±2.2MPa following the combined use of P-S and N. In these cases, the damage to the tooth surface was very small and was equal to or less than damage caused by EDTA. These results demonstrate the usefulness of commercial root canal cleaning agents for dentin surface treatment due to their ability to enhance the bonding of dental resin.

Key words: Root canal cleaning agent, Sodium hypochlorite, Dissolving ability of collagen

INTRODUCTION

It is well known that all commercial bonding agents presently available have extremely limited adhesive strength to unetched tooth surfaces. It is therefore important to examine the causes for this to further improve bonding strength. Dentin poses greater obstacles to adhesive bonding than enamel, and the reports on etching of dentin with various acids either alone or with metal chlorides, i.e., ferric chloride, calcium chloride etc., are currently the most effective methods available. Surface of dentin etched with acids or acidic mixture becomes collagen-rich and heterogeneous. Also, its high water content places severe limitations upon the physical properties of materials which can be used for coupling between dentin and restorative materials.1-3)

Most commercial bonding agents bond to enamel with sufficient strength after treatment with phosphoric acid4,5), to determine the best method of altering the dentin surface so that its chemical composition is closer to that of etched enamel (hydroxyapatite-rich), the dentin surface was treated with chemicals with the ability to dissolve collagen. Among many products,6-8) we selected root canal cleaning agents for experiment.

MATERIALS AND METHODS

Preparation of specimen and adhesive test
Labial surface of freshly extracted bovine incisor was flattened with a silicone carbide paper (JIS #600) under running water, and dried by air spray for 10 s. The resultant surface was
treated with root canal cleaning agent as shown in Table 1. The surface was washed with water-air spray for 30 s and dried with air spray for 15 s. A polyethylene ring, having a cavity 3.6 mm in diameter and 2.0 mm in depth, was fixed on this surface with double-sided adhesive tape. Bonding agent* and composite resin** were applied according to the manufacturer’s instructions. After the curing of the resin, the ring was removed. Resultant specimens were stored in water at 37°C for 24 h. Tensile bond strength was determined by a testing machine# with a cross-head speed of 2.0 mm/min. In each experiment, 10 determinations were made.

**Measurements of surface loss and Knoop hardness**

A sound bovine incisor, embedded in cold curing resin##, was abraded on silicone carbide paper (JIS #100) under running water until a flat dentin surface was obtained. Half of the surface was covered with an adhesive tape. The exposed half was treated with root canal cleaning agents (Table 1) for 60 s. The surface was washed thoroughly with water-air spray for 30 s and dried with air spray for 15 s. Then adhesive tape was removed. Following this procedure, the treated and untreated dentin areas were tested.

A measuring microscope®, with a sensitivity of ±0.5 μm, was used to determine the surface loss of dentin following treatment. The difference of the vertical dimension of microscope between treated and untreated surface was determined as surface loss. This procedure was repeated 10 times along the boundary at intervals of 200–300 μm.

Knoop hardness (Hk) was measured with a 100 g-load and for 30 s using the micro hardness tester@@, as an index of surface loss.

Tensile bond strength, surface loss, and Knoop hardness were analyzed with an analysis of variance and Scheffe’s test for multiple comparisons between the means at the p=0.05

**Table 1** Root canal cleaning agents used.

<table>
<thead>
<tr>
<th>Material</th>
<th>Code</th>
<th>Composition</th>
<th>Manufacturer or distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-C prep</td>
<td>R</td>
<td>10%-Urea peroxide 15%-Ethylene diaminetetraacetic acid</td>
<td>Premier Dental Products Co.</td>
</tr>
<tr>
<td>Hypogen</td>
<td>H</td>
<td>2.5%-Sodium hypochlorite</td>
<td>Premier Dental Products Co.</td>
</tr>
<tr>
<td>Antiformin</td>
<td>A</td>
<td>3%-Sodium hypochlorite</td>
<td>Japan Dental Pharmaceutical Co. Ltd.</td>
</tr>
<tr>
<td>Neo cleaner</td>
<td>N</td>
<td>10%-Sodium hypochlorite</td>
<td>Neo Dental Chemical Products Co. Ltd.</td>
</tr>
<tr>
<td>Oxydolum</td>
<td>O</td>
<td>3%-Hydrogen peroxide</td>
<td>Kenei Seiyaku Co. Ltd.</td>
</tr>
<tr>
<td>Morhonine</td>
<td>M</td>
<td>14.3%-Disodium edetate 0.84%-Cetrimide</td>
<td>Showa Yakuhinkako Co. Ltd.</td>
</tr>
<tr>
<td>PSS</td>
<td>P</td>
<td>70%-Phenolsulfonic acid</td>
<td>Bee Brand-Medico-Dental Co. Ltd.</td>
</tr>
<tr>
<td>PSS neutralization solution</td>
<td>S</td>
<td>9%-Sodium hydrogen carbonate</td>
<td>Bee Brand-Medico-Dental Co. Ltd.</td>
</tr>
</tbody>
</table>

* Clearfil new bond, Kuraray Co., Osaka, Japan
** Clearfil F-II, Kuraray Co., Osaka, Japan
# Autograph DCS-2000, Shimadzu Co., Kyoto, Japan
## Epofix, Struers, Copenhagen, Denmark
@ Nikon measurescope MM-11, Nikon, Tokyo, Japan
@@ DHM-2, Matsuzawa Seiki Co., Tokyo, Japan
level of significance.

*SEM observation*

The flattened dentin surface was prepared and treated with root canal cleaning agents. Dentin specimens were dehydrated in ethanol, dried by a critical point drying apparatus, and coated with gold by an ion coater. Specimens were observed by a scanning electron microscope.

**RESULTS**

*Effect of root canal cleaning agent*

The tensile bond strength of bonding agent to the dentin, treated with root canal cleaning agents, are shown in Fig. 1. In each case, the strength increased compared to that of control. Especially, A and N, both consisting of sodium hypochlorite, and M, consisting of EDTA • 2Na show a remarkable increase in bond strength. SEM micrographs of the dentin surface corresponding to Fig. 1 are shown in Fig. 2. In these micrographs, a slight change was observed except for A, N, and N-O which exhibited smear layers similar to those found on untreated surfaces.

*Optimum concentration of sodium hypochlorite and application time*

In light of the above, the optimum concentration of sodium hypochlorite aqueous solution and its application time to dentin were determined. The higher the concentration, the greater the tensile bond strength until a plateau was reached at 10wt/vol% (Fig. 3). Also, the longer application time was applied, the greater the bond strength was achieved.

---

$^5$ JCPD-5, JEOL, Tokyo, Japan
$^{55}$ IB-3, Eiko Engineering Co. Ltd., Tokyo, Japan
$^+$ S-430, Hitachi Co., Tokyo, Japan
Fig. 2 Scanning electron micrographs of bovine dentin surface treated with root canal cleaning agents. Treatment time: 60 s; O and S, as a counteragent, were applied to the end of foaming.

Fig. 3 Effects of concentration of sodium hypochlorite on the tensile bond strength of bonding agent to bovine dentin, after storage in water at 37°C for 1 day. Treatment time: 60 s; Means connected by bars were not significantly different.
the application time, the greater the tensile bond strength until 60 s (Fig. 4). From these results, the ideal concentration and treatment time of sodium hypochlorite aqueous solution as a dentin treatment agent is considered to be 10 wt/vol% and 60 s, respectively.

Effect of joint use of agents

The manufacturer of R and H recommended that they be used jointly. The effects of the joint use of R and H on the tensile bond strength are shown in Fig. 5. The joint use is more effective than the single use, however the order of use is important. The use of H to dissolve collagen after R as an acid is more effective than the reverse. Moreover, this order of use should be applied to make dentin surface similar to that of acid-etched enamel with respect to the chemical composition, i.e., hydroxyapatite-rich.

The other combinations of root canal irrigants were examined when sodium hypochlorite was used after acid. The results of the joint use of either M or P-S, as an acid, and N, as a sodium hypochlorite, are shown in Figs. 6 and 7, respectively.

The joint use of M and N effectively increased the bond strength, and the treating with M for 30 to 60 s was sufficient. Dentin surface loss was slight, ca. 2 μm, and constant, regardless of treatment time. Also, Knoop hardness did not decrease as the treating time increased.

The joint use of P-S and N also effectively increased tensile bond strength, and treatment with P was enough at 60 s. Although the dentin surface loss increased with treatment time, the loss was small, i.e., 2-3 μm. Regarding Knoop hardness, only a slight etching of the dentin surface was observed following treatment.

In regard to the microstructural change of dentin, SEM micrographs showed very slight changes in the dentin surface after treatment with P-S and N (Fig. 9), and the degree of change was less than that of M and N (Fig. 8).

![Fig. 4](image-url)  
Effect of treatment time of N on the tensile bond strength of bonding agent to bovine dentin, after storage in water at 37°C for 1 day. Means connected by bars were not significantly different.

![Fig. 5](image-url)  
Tensile bond strength of bonding agent to bovine dentin mediated by the joint use of R and H, after storage in water at 37°C for 1 day. Treatment time: 60 s; Means connected by bars were not significantly different.  
H + R: H used prior to R  
R + H: R used prior to H
Fig. 6 Effect of treatment time of M in the joint use of M and N (60 s) on the tensile bond strength of bonding agent to bovine dentin, after storage in water at 37°C for 1 day. Ratio of Knoop hardness of the treated (HK, treated) to control (HK, ground), and the surface loss of dentin were also indicated. Means connected by bars were not significantly different.

Fig. 7 Effect of treatment time of P in the joint use of P-S and N (60 s) on the tensile bond strength of bonding agent to bovine dentin, after storage in water at 37°C for 1 day. S, as a counteragent, was applied to the end of foaming. Ratio of Knoop hardness of the treated (HK, treated) to control (HK, ground), and the surface loss of dentin were also indicated. Means connected by bars were not significantly different.

DISCUSSION

Possibility of root canal cleaning agents as a surface treating agent for enhancing resin bonding strength to dentin

The acid etch technique has been clinically used for restorative and preventive dentistry\textsuperscript{1-3)} since the development of phosphoric acid etching of enamel by Buonocore M. G.\textsuperscript{4,5)} In the case of dentin, the denaturation of collagen following acid etching technique has been problematic,\textsuperscript{9)} although the low bond strength of resin to dentin is thought to be due to various reasons. Therefore, investigation has centered on acids which do not denature collagen,\textsuperscript{10,11)} and/or have some metal chlorides as a mordant to limit the denaturation of collagen.\textsuperscript{8,12-14)}

In this investigation, the intention was to remove the collagen from dentin to enhance
resin bonding during the surface treatment and this could best be achieved by altering the chemical composition of dentin so that it is similar to etched enamel. Collagen, one of major causes of low bond strength to dentin, is thought to be present in small quantities. By removing the collagen, the bond strength to the resultant dentin would increase to that of enamel.

There are many agents which can dissolve the organic compounds. Among them, we selected agents used routinely for root canal cleaning in dental practice. The tensile bond strength of a bonding agent to bovine dentin, treated with these commercial root canal cleaning agents, increased over that of untreated dentin (Fig. 1). In particular, agents composed of sodium hypochlorite (A, N) and EDTA·2Na (M) showed favorable results. In the case of N, the tensile bond strength of bonding agent was superior when they were washed with a water-air spray, than that of followed by neutralization with 3%-hydrogen peroxide. A small quantity of sodium hypochlorite remaining on the treated dentin effectively increases the bond strength.

Traditionally, the removal of the smear layer on the prepared dentin was thought to be
Fig. 9  Scanning electron micrographs of bovine dentin following to the joint use of P-S and N (60 s). S, as a counteragent, was applied to the end of foaming.

sufficient to obtain favorable bonding of resin,\textsuperscript{16} although attempts to remove the smear layer with etching agents were tried in various clinical studies.\textsuperscript{17–21} The SEM micrographs of bovine dentin treated with agents (A, N, and N-O), composed of aqueous sodium hypochlorite, were very similar to that of the control: the smear layer remained on the dentin and morphological change was not observed (Fig. 2). This means the pulp irritation would be to the same level as ground dentin surface. The bacteria in the smear layer on the ground dentin surface is one of the problems encountered clinically.\textsuperscript{20} In this regard, there only a few bacteria were present in the smear layer because of the bacteriolytic effect of sodium hypochlorite.\textsuperscript{6–8,15} So, the surface treatment of dentin with sodium hypochlorite is a very effective method to increase the bond strength of resin with minimum dentinal damage.

Although an experiment was conducted on the joint use of sodium hypochlorite and ultrasound for removing the smear layer of the instrumented root canal dentin,\textsuperscript{22} there are no reports regarding the enhancement of dentin bonding to resin. Therefore, the optimum concentration and treatment time of aqueous sodium hypochlorite was investigated. From the experiment, the optimum concentration was 10 wt/vol\% and treatment time was 30–60
AGENTS HAVING DISSOLVING ABILITY OF COLLAGEN

s (Figs. 3 and 4). These results agree with the dissolving time of collagen (type I) in 10% sodium hypochlorite (aq) 30~60 s.\(^{23}\) With traditional acid etching, the tensile bond strength of a bonding agent to dentin tends to decrease when the optimum condition was not maintained.\(^{14}\) However, with sodium hypochlorite, the resultant tensile bond strength was not influenced with higher concentrations or prolonged treating times. This characteristics are favorable for clinical use.

In this study, EDTA\(^{-2}\)Na (M) also effectively increasing the bond strength. Since Munksgaard and Asmussen\(^{10}\) first used EDTA\(^{-2}\)Na as a surface treating agent followed by a mixture of HEMA and glutaraldehyde, their findings have been confirmed in later reports.\(^{11,12}\) The morphological change of treated dentin was also slight, and it has been reported that the associated tissue reaction is mild.\(^{24}\)

Effect of joint use

In general practice, the joint use of acid and sodium hypochlorite, such as H and R in this study, is recommended for cleaning and enlarging the root canal to obtain a favorable clinical result. With this in mind, the possibility of increasing the bonding strength of resin to dentin was investigated.

First, the effectiveness of joint use of R and H was compared with single use (Fig. 5). The results showed that joint use was more effective than single use, but there was no statistically significant difference except when R was used followed by H. This means that the hydroxyapatite-rich dentin surface is better than the collagen-rich dentin surface for increasing the resin bonding strength. Thus, for best results, acid etching should be carried out first followed by treatment with sodium hypochlorite. This is the opposite of the order used to clean and enlarge the root canal.\(^{25}\) The intent of joint use of R and H for root canal cleaning and enlarging is to make dentin brittle to facilitate the mechanical preparation of the root canal. On the contrary, in the case of surface treatment for enhancing bonding, it is important not to decrease the strength of dentin.

Secondly, the joint uses of M (EDTA\(^{-2}\)Na) and N (Sodium hypochlorite), and of P (phenolsulfonic acid)--S and N were investigated. Both combinations were more effective than the single use, with very little damage to the dentinal surface (Figs. 6, 7, 8 and 9).

Comparison with traditional acid etching

The tensile bond strength of a bonding agent to bovine dentin treated by aqueous sodium hypochlorite was compared to that of traditional acid etching (Fig. 10). As with traditional acid etching, only a slight increase in tensile bond strength was obtained in the case of bovine dentin.

Although sufficient bond strength to bovine dentin was obtained following to the treatment developed in this investigation, no correlation observed between morphological change of tooth structure and resultant bond strength was observed. This is the reason for the choice of treatment agents used in this investigation, sodium hypochlorite, which is more favorable than agents used for traditional acid etching.

Damage of dentin

It is true that the surface treating agents used in bonding system should work with minimum damage. This is particularly important for dentin. In this experiment, the surface loss and Knoop hardness were measured. These results were summarized with tensile bond strength
(Fig. 10). In the acids (B–D), there was no correlation between bond strength and surface loss. In the root canal cleaning agents (E–K), the tensile bond strength was equal to or greater than that treated with citric acid (C). Surface loss and Knoop hardness were similar to those treated by EDTA (D), one of the mildest acids shown in Fig. 10. In addition, it was possible to increase the bond strength without additional damage to the dentin by joint use of a root canal cleaning agent and sodium hypochlorite, which was newly developed for this

Fig. 10  Tensile bond strength of bonding agent to bovine dentin mediated by various surface treating agents, after storage in water at 37°C for 1 day. Ratio of Knoop hardness of treated dentin (HK, treated) to control (HK, ground), and surface loss of dentin were also indicated. Treatment time: 60 s; O and S, as a counteragent, were applied to the end of foaming; Means connected by bars were not significantly different.

A: Control (no treated, #600)  H: N + O
B: 38.5%-Phosphoric acid  I: M
C: 10%-Citric acid  J: P
D: EDTA (0.5M, pH = 7.4)  K: P-S
E: H + R  L: M + N
F: A  M: P-S + N
G: N
Fig. 11 Influence of various surface treating agents on the Knoop hardness and surface loss of bovine dentin. Treatment time: 60 s; O and S, as a counteragent, were applied to the end of foaming.

●: Traditional etchant; ○: Traditional etchant+N; ○: Root canal cleaning agent.

investigation (L and M).

It was also possible to increase the tensile bond strength by joint use of phosphoric or citric acid, traditional etchant, and sodium hypochlorite. However, serious damage to the dentin occurred (Fig. 11). This outcome is undesirable because the bond strength is not affected by surface loss and Knoop hardness of treated dentin surface (Fig. 10).

CONCLUSIONS

In this investigation, we used root canal cleaning agents having sodium hypochlorite to dissolve collagen, and a mild acid to dissolve hydroxyapatite. The result was a hydroxyapatite-rich dentin surface which facilitates bonding.

The following results were obtained:

1. All commercially available root canal cleaning agents were effectual for increasing the bond strength to dentin. Especially, aqueous sodium hypochlorite, N, appeared to offer greater strength with only slight damage to the dentin.
2. The removal of a smear layer from the dentin surface was not an essential factor for the increase of bond strength.
3. A treatment time of 30–60 s was enough to obtain a favorable bonding strength. Longer treatment times was effectual but was also harmful to the tooth substance.
4. The joint use of acids and sodium hypochlorite achieved the best results. In this case, acid should be applied prior to sodium hypochlorite.
ACKNOWLEDGMENT

This study was supported in part by Grant-in-Aid for Scientific Research (B) 01480449 from the Ministry of Education, Science and Culture of Japan.

REFERENCES

21) Eick, J. D. and Welch, F. H.: Dentin adhesives -Do they protect the dentin from acid etching?,
Quintessence International 17 (9): 533-544, 1986.


25) Directions for use of premier "R-C PREP"


データが完成した。これらはCAMのための基礎データとなり得るものである。

コラーゲン溶解能を有する根管清掃剤の象牙質とレジンとの
接着性を向上させる被着面処理への応用

田仲将郎、中井宏之
岡山大学歯学部歯科室理工学講座

著者らは、象牙質に対する接着性改善の為に、エナメル質に対する接着性が良好であることに着目し、象牙質被着面をエナメル質と化学的に同様のハイドロキシアパタイトリッチの状態とすることを試みた。この目的を達成する為にコラーゲン溶解能を持つ化合物として知られている根管清掃剤による歯面処理を試みた。

その結果は、酸系の根管清掃剤と次亜塩素酸ナトリウム系の根管清掃剤との併用が効果的であり、MとNの系で牛歯象牙質に10.7±3.9MPa、P-SとNの系で8.8±2.2MPaで良好な引張り接着強さを示した。さらに、これら根管清掃剤による象牙質処理面のSurface lossは従来の代表的な酸処理に較べて極めて少なく、現在、最も脱灰量の少ない部類に属するEDTAと同等またはそれ以下であった。また、処理被着面の硬度がほとんど低下していないことも大きな特徴であった。以上の結果は、根管清掃剤が歯科用レジンの象牙質に対する接着性改善に関する歯面処理剤として有効であることを示した。

酸性溶液中におけるヒドロキシアパタイトとフッ化物との
反応に関するコンピュータシミュレーション

三浦宏子、荒木吉馬1、大野弘機1
東日本学園大学歯学部口腔衛生学講座
1 東日本学園大学歯学部歯科室理工学講座

合成ヒドロキシアパタイト（HAp）とフッ化物溶液との沈殿生成反応系について、コンピューターシミュレーションによる反応解析を行った。今回用いた反応系は、0.5～2mMのフッ化カリウムを含む0.05～0.02Mの酢酸緩衝液中に、HApを過剰に加えた系である。反応過程におけるHApの溶解量と、カルシウム塩の沈殿量ならびに系内の化学種の活量を計算した。その結果、フッ化物溶液をHApに反応させると、まずフッ化カルシウムが優先的に析出する。その後、さらにHApの溶解が進むと、FApに関してイオン活動度値がFApの溶解度値を超す臨界pH点において、FApが析出し始める。それについて、既に沈殿していたCaF2は溶解する。

この臨界pH值は、緩衝液自体のpHが低いものほど高くなるが、フッ素イオン濃度の影響は少なかった。系において、CaF2よりもFApを優先的に析出させるためには、溶液中にあらかじめリン酸イオンを加えて、FApのイオン活動度値を高めておくことが必要であることが明らかになった。