Comparison of the Occluding Ability of Dentinal Tubules with Different Morphology between Calcium Phosphate Precipitation Method and Potassium Oxalate Treatment

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The aim of this study was to evaluate the occluding ability of calcium phosphate precipitation (CPP) method and potassium oxalate treatment when each method was applied to dentin disks with different surface morphology. Occluding ability was observed by scanning electron microscopy. Irrespective of the diameter of the dentinal tubules, the CPP method showed a consistent occluding ability for dentinal tubules at the dentin surface, and that the depths of the precipitate formed in the dentinal tubules by CPP method were not significantly different. In contrast, the occluding ability of potassium oxalate treatment was reduced with increasing diameter of the dentinal tubules. However, the reduction of the occluding ability of potassium oxalate treatment was more markedly affected by the demineralization of dentin surface. Since the CPP method showed a consistent occluding ability irrespective of the diameter of the dentinal tubules, it is suggested that the CPP method would be a useful means for treating dentin hypersensitivity.

Key words : Calcium phosphates, Dentinal tubules, Dentin hypersensitivity

INTRODUCTION

We have reported on a calcium phosphate precipitation (CPP) method which occluded open dentinal tubules with apatitic mineral1-6. The CPP method was based on the significant differences in solubility of calcium phosphate mineral at different pH values. In other words, larger amounts of calcium phosphate could be dissolved in solutions with lower pH whereas virtually no calcium phosphate could be dissolved in neutral or basic solutions. When acidic CPP solution (1 mol/L CaHPO4·2H2O dissolved in 2 mol/L H3PO4) was applied to a dentin surface with a cotton swab and neutralized with a post-treatment solution (1 mol/L NaHCO3 with 0.3 mol/L NaF) using a cotton swab, the patent dentinal tubules — which are typical for dentin surface subject to dentin hypersensitivity — were occluded completely and homogeneously not only on the surface of the dentinal tubules, but also inside the dentinal tubules. The occluding depth of CPP method was approximately 10 μm from the dentin surface.

For the purpose of evaluating the occluding ability of the CPP method through our in vitro study, simulated hypersensitive dentin disks etched with 50% citric acid for two minutes were prepared according to a previous method7. However, the condition of the dentin surface, i.e., the diameter of the dentinal tubules, appeared to be different between severely hypersensitive dentin and the early stage of dentin hypersensitivity8. In fact, our previous intraoral study demonstrated that dentinal tubules became widely open by demineralization with plaque when no plaque control was exercised9. The opening ratio of the dentinal tubules after three weeks increased threefold compared with the initial value in the non-plaque control group. In contrast, dentinal tubules were gradually obturated by precipitation of calcium and phosphate when plaque control was exercised through daily brushing or chemical removal of plaque using chlorhexidine9. In general, a solution can penetrate deeper with increase in radius of a tube following the Poiseuille-Hagen law10. The CPP method formed apatitic precipitate in dentinal tubules as a result of a chemical reaction between the CPP and post-treatment solutions. Therefore, we should evaluate whether the CPP method could obtain similar occlusion of dentinal tubules even though the CPP method is applied to small diameter dentinal tubules.

Except for the diameter of dentinal tubules, teeth components are not always constant on all hypersensitive dentin teeth. Acidic beverages and fruit juices cause enamel erosion and consequently evoke dentin hypersensitivity. In such a case, the amount of inorganic mineral of the tooth surface, i.e., calcium and
phosphate, may be reduced by demineralization. This means that with hypersensitive teeth, the components of the dentin surface and subsurface would be different from those of sound dentin. Components of teeth, especially the amount of calcium, significantly influence the occluding ability of potassium oxalate treatment. Potassium oxalate treatment formed calcium oxalate crystals in dentinal tubules, and so dentin permeability was significantly reduced\(^1\). Moreover, 3% monohydrogen-monopotassium oxalate solution was used to increase the occluding ability following 30% dipotassium oxalate solution. These two kinds of potassium oxalate treatment formed crystals of different sizes in dentinal tubules. The 3% monohydrogen-monopotassium oxalate solution is an acidic solution — therefore, it would increase calcium oxide formation by supplying calcium ions from the tooth by demineralizing the tooth\(^2\). Thus, the amount of available calcium ions in hypersensitive teeth would markedly affect the formation of calcium oxalate crystals after potassium oxalate application.

It is indispensable for an ideal treatment of dentin hypersensitivity to show consistent occluding ability regardless of the diameter of dentinal tubules and the condition of the dentin i.e., mineral contents. The aim of this study was to evaluate the occluding ability of CPP method and potassium oxalate treatment when these treatment methods were applied to dentin disks with different surface morphology.

MATERIALS AND METHODS

Preparation of CPP, post-treatment and potassium oxalate solutions

CPP solution was prepared as described previously\(^1\). In brief, reagent grade 1 mol/L dicalcium phosphate dihydrate (CaHPO\(_4\cdot2\)H\(_2\)O; Nacalai Tesque, Kyoto, Japan) was dissolved in 2 mol/L H\(_2\)PO\(_4\) solution at room temperature to prepare CaHPO\(_4\cdot2\)H\(_2\)O-saturated CPP solution. Clear solutions ([Ca]=1 mol/L; [PO\(_4\)]\(_4\)=3 mol/L) were filtered through 0.22 μm Millex-GS filter assemblies (Millipore Corp., Bedford, MA) and kept in closed bottles at room temperature. Post-treatment solution was prepared from reagent grade 1 mol/L NaHCO\(_3\) aqueous solution with 0.3 mol/L NaF as described previously\(^3\). In addition, 30% dipotassium oxalate solution and 3% monohydrogen-monopotassium oxalate solution were prepared from reagent grade chemicals (Kanto Chemicals, Tokyo, Japan).

Preparation and treatment of dentin disks

Dentin disks were obtained from healthy adults with informed consent, through caries-free human molars extracted for periodontal reasons at the Tokushima University Dental Hospital. Each tooth was sectioned horizontally below the cementoenamel junc-
tion. To prepare the disks from the middle of the coronal dentin, enamel was removed with a high-speed water-cooled handpiece, followed by sectioning with a low-speed water-cooled diamond saw (Buehler Ltd., Evanston, IL) to approximately 1.5 mm thickness.

Dentin disks were divided into four pretreatment groups as follows for the purpose of preparing simulated hypersensitive dentin with open dentinal tubules.

Group 1: Smear layer was treated with sonicatation (Shofu, Kyoto, Japan). The disks were immersed in distilled water and then sonicated (43 kHz) for 20 minutes. This procedure only removed the smear layer and smear plug without demineralization. Therefore, the dentinal tubules kept their original diameter.

Group 2: Smear layer was treated by immersion in 0.5 mol/L EDTA (pH=7.4) for two minutes\(^3\).

Group 3: Smear layer was treated by immersion in 6% citric acid for two minutes\(^3\).

Group 4: Smear layer was treated by immersion in 50% citric acid for two minutes\(^7\).

The disks were then washed with distilled water and dried using a three-way syringe. Each dentin disk pretreated by one of the above procedures was divided through the center into almost two equal parts. One part was used for the experiment (treated by the CPP method or potassium oxalate solution) and the other served as a control.

The CPP method was applied to each pretreated dentin disk. In brief, CPP solution was applied to the dentin disk with a cotton swab for 30 seconds, followed by drying gently with a cotton swab. Following this, post-treatment solution was applied to the dentin disk with a cotton swab for one minute. These procedures were repeated twice to increase the occlusion depth of the precipitate in the dentinal tubules.

As for the potassium oxalate treatment, 30% dipotassium oxalate solution was applied to the dentin disk with a cotton swab for three minutes. In addition, two kinds of potassium oxalate treatment were applied to the dentin disk. First, 30% dipotassium oxalate solution was applied to the dentin disk with a cotton swab for three minutes followed by application of 3% monohydrogen-monopotassium oxalate solution for three minutes.

After the CPP or potassium oxalate treatment, the tooth was washed with distilled water for one minute and dried with a critical point dryer (Hitachi Co., Tokyo, Japan).

SEM observation

The occluding ability of the CPP method or potassium oxalate treatment against several diameters of dentinal tubules was evaluated by scanning electron microscopic (SEM; S-700, Hitachi Co., Tokyo, Japan)
observations. Specimens obtained following the above procedures were fractured longitudinally into equal halves at the center. In brief, a groove was prepared on the pulpal side of the dentin disk, with fine diamond burs used from the palatal to the facial surface. The tooth, held between thumb and forefinger, was then sectioned in half longitudinally by pressure exerted on the medial and distal surfaces.

After the specimens were gold-coated, the surface and fractured surface were observed by SEM. Control specimens were observed by SEM too, and then the average diameter of dentinal tubules was calculated using SEM photographs. Two adjacent areas of the same disk were used to evaluate the occluding ability for accuracy and symmetry: one was the test surface, the other was a control following a previous method. Each pretreatment group used at least 15 dentin specimens. The depth of the precipitate formed by the CPP method was evaluated in at least 30 dentinal tubules obtained from at least five independently treated specimens.

**Percentage of open tubules**

Occluding ability of the CPP method and potassium oxalate treatment were assessed quantitatively by computer image analysis software using SEM photographs. After SEM observation, SEM photographs (×4000 magnification) were scanned into a computer using a scanner, and then the area of the open dentinal tubules was marked using the program, Adobe Photoshop 4.0.1 (Adobe ver 4.0.1 for Power PC). Then NIH image (ver 1.62) was used to measure the area of open dentinal tubules per unit area. We defined and calculated the percentage of open tubules per unit area as an index of dentinal tubule occlusion, as shown in Equation (1).

\[
\text{Percentage of open tubules} = \frac{\text{Area of open dentinal tubules}}{\text{Unit area}} \times 100 \quad \text{Eq (1)}
\]

The percentage of open tubules obtained from SEM photographs was the average value of at least 10 different images.

**Statistical analysis**

For statistical analysis, one way factorial ANOVA and Fisher's PLSD method (used as a post-hoc test) were performed using the program, Stat View 4.02 (Abacus Concepts Inc., Berkeley, CA). Statistically significant differences were set at p<0.05.

**RESULTS**

Typical SEM pictures of the dentin surface and the longitudinally sectioned surface after pretreatment are shown in Fig.1. When the dentin disk was pretreated with sonication, the dentinal tubules kept their original diameter (Figs.1(a) and 1(e)). With EDTA pretreatment, the dentinal tubules were slightly demineralized (Figs.1(b) and 1(f)). In contrast, after pretreatment with 6% (Figs.1(c) and 1(g)) or 50% citric acid (Figs.1(d) and 1(h)), the diameter of dentinal tubules increased and the dentinal tubules exhibited funnel-like shape near the dentin surface.

SEM micrographs of the surface and fractured surface of dentin specimens after CPP treatment are shown in Fig.2. Dentinal tubules at the dentin surface were occluded completely with the precipitate irrespective of pretreatment method. Indeed, the CPP method occluded open dentinal tubules similar to a previous study, even if the CPP method was applied to small-diameter dentinal tubules pretreated with ultrasonic irrigation (Figs.2(a) and 2(e)). The precipitate occluded not only on the surface of the dentin disk, but also within the dentinal tubules. Depth of the precipitate formed by the CPP method was approximately 10 μm from the dentin surface, and no significant differences were observed among the four pretreatment groups.

In Fig.3, SEM photographs after treatment with 30% dipotassium oxalate solution are shown. With this treatment, relatively large-size, plate-like crystals were precipitated in dentinal tubules when the specimens were pretreated with sonication or EDTA. In addition, the crystalline precipitate could be observed in dentinal tubules in longitudinal sections. However, the dentinal tubules were not occluded completely when the treatment was applied to demineralized dentin (pretreatment with 6% or 50% citric acid). In longitudinal sections, precipitate could only be observed at the deep part of dentinal tubules with small amount of precipitate in the dentinal tubules near the dentin surface.

Fig.4 shows the SEM photographs after treatment with 30% dipotassium oxalate followed by 3% monohydrogen-monopotassium oxalate. With this treatment, smaller precipitates than those of 30% dipotassium oxalate treatment were formed. However, this two-step oxalate treatment applied to dentin disks after pretreatment with 6% or 50% citric acid could not occlude the orifice of dentinal tubules at the dentin surface similar to the result yielded by 30% dipotassium oxalate treatment.

Table 1 shows the percentage of open tubules before and after the CPP method or potassium oxalate treatment. The percentage of open tubules for pretreatment with sonication and EDTA were relatively small, but increased with the concentration of citric acid. The percentage of open tubules after treatment with CPP method was significantly reduced in all pretreatment groups. In addition, both potassium oxalate treatments reduced the percentage of open tubules when the disks were pretreated with isotonic irrigation or EDTA. However, for pretreatment with 6% or 50% citric acid, the percentage of open tubules
Fig. 1 Typical scanning electron micrographs of the surface and fractured surface of dentin specimens after pre-treatment: (a) (e) After ultrasonic irrigation for 20 min; (b) (f) After treatment with 0.5 mol/L EDTA for 2 min; (c) (g) After etching with 6% citric acid for 2 min; (d) (h) After etching with 50% citric acid for 2 min. Bar represents 5 μm.

Fig. 2 Typical scanning electron micrographs of the surface and fractured surface of dentin specimens after CPP treatment. The CPP method applied to the dentin disk: (a) (e) after pretreatment with ultrasonic irrigation for 20 min; (b) (f) after pretreatment with 0.5 mol/L EDTA for 2 min; (c) (g) after pretreatment with 6% citric acid for 2 min; (d) (h) after pretreatment with 50% citric acid for 2 min. Bar represents 5 μm.
Fig. 3  Typical scanning electron micrographs of the surface and fractured surface of dentin specimens after 30% dipotassium oxalate treatment. The 30% dipotassium oxalate applied to dentin disk: (a)(e) after pretreatment with ultrasonic irrigation for 20 min; (b)(f) after pretreatment with 0.5 mol/L EDTA for 2 min; (c)(g) after pretreatment with 6% citric acid for 2 min; (d)(h) after pretreatment with 50% citric acid for 2 min. Bar represents 5μm.

Fig. 4  Typical scanning electron micrographs of the surface and fractured surface of dentin specimens after 30% dipotassium oxalate followed by 3% monohydrogen potassium oxalate. Two kinds of potassium oxalate solutions applied to dentin disk: (a)(e) after pretreatment with ultrasonic irrigation for 20 min; (b)(f) after pretreatment with 0.5 mol/L EDTA for 2 min; (c)(g) after pretreatment with 6% citric acid for 2 min; (d)(h) after pretreatment with 50% citric acid for 2 min. Bar represents 5μm.
Table 1 The percentage of open tubules before and after being treated with the CPP method or potassium oxalate treatment

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Before (%)</th>
<th>CPP (%)</th>
<th>KOx (%)</th>
<th>KOx + KHOx (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonication</td>
<td>17.9 (5.6)</td>
<td>1.0 (0.1)</td>
<td>0.0 (0.0)</td>
<td>2.2 (0.1)</td>
</tr>
<tr>
<td>EDTA</td>
<td>18.4 (0.1)</td>
<td>0.4 (0.3)</td>
<td>0.1 (0.1)</td>
<td>0.3 (0.4)</td>
</tr>
<tr>
<td>6% citric acid</td>
<td>25.2 (7.9)</td>
<td>0.1 (0.2)</td>
<td>16.7 (4.0)</td>
<td>19.0 (5.8)</td>
</tr>
<tr>
<td>50% citric acid</td>
<td>40.8 (3.3)</td>
<td>1.9 (4.6)</td>
<td>36.7 (3.9)</td>
<td>28.6 (4.2)</td>
</tr>
</tbody>
</table>

( ): Standard deviation.
KOx: Treatment with 30% dipotassium oxalate for 3 min.
KOx + KHOx: Treatment with 30% dipotassium oxalate for 3 min followed by application of 3% monohydrogen-monopotassium oxalate for 3 min.

Table 2 Diameter of dentinal tubules after pretreatment and depth of precipitate in dentinal tubules formed by the CPP method

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Diameter of dentinal tubules (μm)</th>
<th>Precipitate depth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonication</td>
<td>0.8 (0.2)</td>
<td>9.2 (2.4)*</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.9 (0.3)</td>
<td>10.4 (2.2)*</td>
</tr>
<tr>
<td>6% citric acid</td>
<td>2.3 (0.3)</td>
<td>10.7 (2.3)*</td>
</tr>
<tr>
<td>50% citric acid</td>
<td>2.9 (0.6)</td>
<td>9.7 (2.3)*</td>
</tr>
</tbody>
</table>

( ): Standard deviation.
*No significant differences were observed among four pretreatment groups.
N=30

after potassium oxalate treatment was only reduced slightly.

Table 2 summarizes the average diameter of dentinal tubules after pretreatment, as well as the depth of the precipitate formed in dentinal tubules by CPP method after each pretreatment. Basically, the depths of the precipitate did not differ significantly among the various pretreatment groups and were not influenced by the diameter of the dentinal tubules.

DISCUSSION

This study clearly demonstrated that the CPP method could occlude open dentinal tubules homogeneously and completely regardless of the diameter of dentinal tubules. In contrast, both potassium oxalate treatments showed different occluding ability of dentinal tubules, especially for large-diameter dentinal tubules, which remained open after potassium oxalate treatment. The reduced occluding ability with potassium oxalate treatment was markedly affected by the degree of demineralization of the tooth rather than the diameter of dentinal tubules. Potassium oxalate treatment occludes open dentinal tubules with formation of calcium oxalate as a result of reaction with calcium in tooth. Therefore, the amount of calcium oxalate precipitate decreases when there are insufficient calcium ions in the tooth.

The reason for the reduction of precipitate could be explained by SEM observation. Pretreatment with sonication or EDTA removed the smear layer and opened the dentinal tubules with or without a little demineralization. As a result, with barely any change in the available calcium ions in the tooth, the amount of calcium oxalate formed in dentinal tubules was not reduced too. In contrast, when dentin disks were demineralized with 6% or 50% citric acid, no precipitates were observed at funnel-like shaped dentinal tubules near the dentin surface, although large amounts of precipitate were formed at the deep part of the dentinal tubules not subject to demineralization. The two-step potassium oxalate treatment occluded dentinal tubules with precipitates smaller than those of 30% dipotassium oxalate. This two-step treatment was used to increase the occluding ability of dipotassium oxalate treatment by supplying calcium ions from the tooth — due to demineralization of the tooth by acidic 3% monohydrogen-monopotassium oxalate. However, it was difficult to obtain complete occlusion of dentinal tubules at the dentin surface even when two kinds of potassium oxalate solution were applied to demineralized dentin. In this study, human extracted teeth were used to evaluate occluding ability — therefore, dentinal tubules are not filled with dentinal fluids. The occluding ability of potassium oxalate treatment may be increased when this method is used with vital teeth due to the supply of calcium ions from dentinal fluids. However, the amount of precipitate formed in dentinal tubules after potassium oxalate treatment was similar in our previous in vitro and in vivo studies using the vital teeth of beagle dogs. Therefore,
supply of calcium ions from dentinal fluids has a low effect on the amount of precipitate formed.

The average diameter of dentinal tubules in hypersensitive teeth was reported as 0.83 ± 0.38 (mean ±SD) μm\(^2\). This reported value was similar to the diameter of dentinal tubules after pretreatment with sonication or EDTA in this study. Thus, potassium oxalate treatment would be effective for most cases of hypersensitive dentin treatment. However, the condition of the dentin surface appears to be different between severely hypersensitive dentin and the early stage of dentin hypersensitivity. In fact, our previous intraoral study showed that dentinal tubules became widely open by demineralization with plaque when no plaque control was exercised\(^9\). The opening ratio of the dentinal tubules increased threefold compared with the initial value in the non-plaque control group after three weeks. In contrast, dentinal tubules were gradually obturated by precipitation of calcium and phosphate when plaque control was exercised through daily brushing or chemical removal of plaque using chlorhexidine\(^6,16\). Therefore, the condition of plaque control markedly affected the pathology of hypersensitive dentin\(^2\). In addition, acidic beverages remove the smear layer and open dentinal tubules\(^{7}\). Most acidic beverages, such as sports drinks, contain citric acid. For this reason, the tooth surface was demineralized through pretreatment with 6% citric acid in this study. In such a case, potassium oxalate treatment could not completely occlude the open dentinal tubules. For cases of more severe dentin hypersensitivity, dentinal tubules are widely opened by demineralization with plaque and plaque products — similar to the observation after pretreatment with 50% citric acid in this study. In contrast, the diameter of dentinal tubules at the early stage of dentin hypersensitivity may be relatively small. Therefore, it is necessary for the ideal treatment of dentin hypersensitivity to occlude dentinal tubules completely regardless of the diameter of dentinal tubules and the condition of the tooth.

In our previous study, dentin permeability was calculated before and after potassium oxalate treatment following Pashley's method\(^{10}\). Dentin permeability was significantly reduced although the dentin disks were pretreated with 50% citric acid for two minutes\(^2,3\). Reduction of dentin permeability was due to occlusion of the deep part of dentinal tubules, while little of the precipitate could be observed at the dentin surface and subsurface. From the results of this study, the most suitable clinical case for potassium oxalate treatment appeared to be at the beginning of dentin hypersensitivity without marked tooth demineralization. Moreover, preventive treatment for dentin hypersensitivity immediately after periodontal surgery would be useful since root surface is covered with a smear layer which contains an abundance (putative) of inorganic minerals. The existence of the smear layer on the dentin surface was reported to be effective for potassium oxalate treatment to form calcium oxalate crystals\(^9\). To increase the occluding ability of potassium oxalate treatment even when applied to demineralized dentin, the supply of calcium ions seems to be an effective means\(^{20}\). For example, a calcium solution applied to dentin before or after potassium oxalate treatment may be expected to form large amounts of calcium oxalate precipitates.

The CPP method occludes open dentinal tubules with apatitic minerals as a result of the reaction of CPP solution and post-treatment solution in dentinal tubules. The CPP solution and post-treatment solution penetrate into dentinal tubules by capillary action. In general, penetration of a solution through a tube depends on the radius of a tube following the Poiseuille-Hagen law. The average diameter of dentinal tubules was approximately 0.8 μm to 2.9 μm in the present study. However, the diameter of dentinal tubules did not affect the occluding ability of the CPP method, especially the depth of the precipitate.

Based on the results of this study, the CPP method seemed to be a potentially promising treatment for dentin hypersensitivity, be it at the early stage or for severely hypersensitive dentin. This is because the method could occlude dentinal tubules regardless of the diameter and condition of the dentinal tubules. Indeed, clinical use of the CPP method await further in vitro and in vivo evaluations — which will be based on the findings of this study.

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