Summary This study evaluated the effects of the short-term use of a dentifrice containing nano-sized carbonate apatite (n-CAP) on the occlusion of the dentinal tubules using a scanning electron microscope (SEM) and an image analyser in vitro. One hundred human dentine specimens were wet ground with a silicone carbide papers and etched with 6% citric acid for 1 min to allow complete opening of the dentinal tubule. Specimens showing complete opening tubules were used as the baseline. The specimens were divided randomly into five groups: G1: 0% n-CAP, G2: 5% n-CAP, G3: 10% n-CAP, G4: 20% n-CAP and G5: 10% strontium chloride (SrCl2). Five specimens from each group were brushed by applying 50, 100, 250 and 500 strokes, respectively. All the specimens were evaluated by a SEM (>3000), and the degree of occlusion of the dentinal tubules was quantified using an image analyser. The results were analysed by one-way anova and a Tukey’s test using the spss 12.0 statistical package program. The dentifrice containing 20% n-CAP for 50 strokes, which indicated 2-day use, showed the highest tubular occlusion than the other groups ($P<0.05$). Moreover, this group showed 79.5% and 77.4% less open tubular area than the baseline and 0% n-CAP group, respectively. The groups containing various concentrations of n-CAP showed significant differences in the SrCl2 group after tooth-brushing for 500 strokes, which indicated 17-day use. According to this examination of the short-term use of desensitizing dentifrices in vitro, the dentifrice containing 20% n-CAP was the most effective in occluding the dentinal tubules. Keywords: dentinal tubule occlusion, nano-carbonate apatite, desensitizing dentifrice

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Introduction

According to the Brännström’s hydrodynamic mechanism, exposed dentin with patent tubules allows the movement of tubule fluid which leads to dentine sensitivity (1). Variations in the dentin hypersensitivity symptoms occur according to the extent of opened dentinal tubules. Exposed dentin has been shown to have wider and more number of dentinal tubules than non-sensitive dentin, which is mostly covered by a smear layer (2, 3). Therefore, an occlusion of patent dentinal tubules would be an effective means of treating dentine hypersensitivity. Various chemical agents have been used to occlude dentinal tubules and decrease the hypersensitivity symptoms (4–6). Desensitizing dentifrices containing active ingredients such as calcium carbonate, strontium chloride (SrCl2), silica and potassium have therapeutic potential for partially or completely occluding the dentinal tubules (7). These products are aimed at reducing the hypersensitive symptoms through daily tooth-brushing at home. A dentifrice containing SrCl2 is a representative desensitizing dentifrice. The main mechanism of this product is known to close the dentinal tubules.

Hydroxyapatite (HAP) is one of the most biocompatible and bioactive materials (8) on account of its similarity to the mineral compositions found in teeth. Although HAP is widely used as an osteo-conductive biomaterial, carbonate apatite (CAP) is more similar to the inorganic component of teeth or bones (9). Therefore, the CAP has recently been used as a bone substitute and a coating material on dental implants.
because of its superior biocompatibility. CAP combined with nano-technology to increase the surface area by the extremely fine particle size can be expected to show good physical properties. It is known that nano-sized CAP (n-CAP) have a much higher solubility and a more neutral pH than previous micro-sized HAP. Therefore, n-CAP might also have potential use in desensitizing dentifrices.

The aim of this study was to evaluate the occluding effects of the dentinal tubules in short-term use of dentifrices containing n-CAP with a scanning electron microscope (SEM) and an Image analyser in vitro.

Materials and methods

Specimen preparation

Recently, extracted human molars without caries were collected and stored in a 0-1% thymol solution at 4°C. The teeth were sectioned horizontally below the cemento-enamel junction using a diamond wheel disc, and the dentin in the root portion was used. One hundred dentin specimens were embedded with an acrylic resin into a teflon mold (19 mm×12 mm×8 mm). Each dentin surface was wet ground using silicone carbide papers (600–2000 grits) on a polishing machine to expose the dentin surface. The dentin specimens were etched with 6% citric acid for 1 min to completely open the dentinal tubule and ultrasonicated in distilled water for 30 min to remove the residual smear layer. The specimens were placed in distilled water until they were required for treatment.

Experimental groups

The specimens were randomly divided into the following five groups, each containing twenty specimens; negative control dentifrice (group 1: 0% n-CAP), experimental dentifrices (group 2: 5% n-CAP, group 3: 10% n-CAP and group 4: 20% n-CAP) and positive control dentifrice (group 5: 10% SrCl2). All experimental dentifrices contained 1000 ppm fluoride and 8% silica as an abrasive. The positive control dentifrice also contained 10% calcium carbonate as an abrasive without fluoride. The concentration of the abrasive agents used in this study was lower than in general dentifrice because these experimental dentifrices were targeted for dentin hypersensitivity patients.

Treatments and analysis

Because the primary purpose of this study was to evaluate the short-term effects of the experimental dentifrices, the brushing frequency was determined from 2 to 17 days of use. Five specimens from each group were brushed with a V8 Cross Brushing Machine (Sabri Enterprises, Downers Grove, IL, USA) for 50, 100, 250 and 500 strokes respectively. Tooth-brushing was performed at a force produced by 150 g weight attached to the brush head and at a rate of 50 back and forth strokes per minute. The dentifrice slurries were prepared as 20 g of the dentifrice in 80 mL of distilled water. The specimens were rinsed with distilled water after tooth-brushing. After drying in a 60°C oven for 24 h, each specimen was mounted on metal holders and coated with gold. In this study SEM (S-800 Hitachi Ltd., Tokyo, Japan) was used to observe the surface of all specimens, and an area of interest for the SEM images was selected horizontally in three parts from the center area of each specimen.

The degree of occlusion of the dentinal tubules was quantified using an image analyser (Image-Pro PLUS, v 6.0, Media Cybernetics, Silver Spring, MD, USA). Image analysis was used to measure all the opened tubules within the SEM image of each specimen surface. Instead of counting the number of opened tubules as in a previous study (3), this study measured the opened area of each dentinal tubule. The SEM images were saved as JPEG files for image analysis. Each image was calibrated using the 1 μm scale bar in the SEM image to quantify the measured area. The polygon tool in the major tool bar was used to measure the area of dentinal tubules. This software can automatically draw the highlighted outline of a dentinal tubule from the difference in grey pixels between the dentinal tubule and the outer area, and then calculate the mean total tubules area.

The results between the groups were analysed by one-way anova and Tukey’s post hoc analysis using the spss 12.0 statistical package program (SPSS Inc., Chicago, IL, USA). Two-way anova was performed to determine if there was a significant interaction between the strokes and groups.

Results

Table 1 shows the changes in the dentinal tubules after tooth-brushing with the five dentifrices and the mean
area of the occluded tubules in each group. Before tooth-brushing, the mean dentinal tubule area in the etched baseline specimens (n = 5) was 5.26 μm². On the other hand, the size of the dentinal tubules decreased after tooth-brushing using all the dentifrices. Two-way anova showed a significant interaction between the brushing strokes and groups on tubule occlusion (Table 1, P < 0.05) and there were statistically significant differences between the groups (P < 0.05). The 20% n-CAP group for 50 strokes, which indicated 2-day use showed the highest level of tubule occlusion compared with the other groups (P < 0.05) with 79.5% and 77.4% less open tubular areas than the baseline and 0% n-CAP groups, respectively. The 10% n-CAP group had the highest occlusion effect and there was a significant difference in the SrCl₂ group after 100 strokes (P < 0.05). However, there was no statistically significant difference between the groups after tooth-brushing for 250 strokes. After 500 brushing strokes which indicated 17-day use, there were significant differences between the SrCl₂ group which was a positive control group and the experimental groups containing 5–20% n-CAP (P < 0.05). The n-CAP groups from 5% to 20% showed 52.7–57.6% less open tubular area than the baseline tubular areas after 500 strokes.

Figure 1 shows SEM images of the dentinal tubules after tooth-brushing with the various dentifrices for 50 strokes. The baseline specimen which was etched with 6% citric acid for 1 min showed a higher number and distinct widening of the dentinal tubules (Fig. 1a). In contrast, the dentinal tubules were occluded and fewer in number after brushing with the dentifrices (Fig. 1b–f). In particular, the specimen treated with the dentifrice containing 20% n-CAP showed complete occlusion of the dentinal tubules (Fig. 1e). In addition, the surface of the specimens was covered by very small particles. High magnification SEM images (×50000) confirmed that small particles covered the dentin specimens treated with the 20% n-CAP dentifrice (Fig. 2b). However, the 0% n-CAP group showed no particles on the specimen surface (Fig. 2a). SrCl₂ of the positive control group also showed small particles covering the surface (Fig. 2c). After tooth-brushing for 500 strokes, the n-CAP groups showed closed tubules in relation to the n-CAP concentration. However, the SrCl₂ group still showed opened dentinal tubules (Fig. 3).

**Discussion**

Most people with dentin hypersensitivity prefer to use a desensitizing dentifrice because tooth-brushing is one of the easiest methods in a home care system. Some studies have reported that dentifrices containing SrCl₂ (3), calcium phosphates (6, 10), oxalate (11) and fluorides (12) can increase the level of dentinal tubule occlusion. Dentifrice abrasives, such as calcium carbonate, silica and dicalcium phosphate can also have a closing effect of the dentinal tubules (7, 13). This phenomenon can occur directly by closing the tubules with an abrasive or indirectly through the formation of a smear layer during tooth-brushing (4, 14, 15).

Clinically, dentifrices containing HAP are used to treat dentine hypersensitivity because of its chemical similarity to enamel. However, until now there have not been any trials applying n-CAP to desensitize dentifrices as it is more similar to the inorganic component of teeth or bones than HAP. The n-CAP is known to have a much higher solubility and reactivity than HAP. In addition, the nano-sized particles have a

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**Table 1. Comparisons of the dentinal tubule area after tooth-brushing with each dentifrice (unit: μm²)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>50</th>
<th>100</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0% n-CAP</td>
<td>20</td>
<td>4.77 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.51 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.35 ± 1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.67 ± 0.80&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 5% n-CAP</td>
<td>20</td>
<td>4.18 ± 1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.29 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.44 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23 ± 1.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 10% n-CAP</td>
<td>20</td>
<td>4.39 ± 1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.58 ± 1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 20% n-CAP</td>
<td>20</td>
<td>1.08 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.57 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.64 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.38 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 10% SrCl₂</td>
<td>20</td>
<td>3.17 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.15 ± 1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.28 ± 1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.25 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P-value: <0.001, <0.01, <0.05, <0.02, <0.03, <0.04, <0.08, <0.10, <0.12, <0.14, <0.16, <0.18, <0.20, <0.22, <0.24, <0.26, <0.28, <0.30, <0.32, <0.34, <0.36, <0.38, <0.40, <0.42, <0.44, <0.46, <0.48, <0.50, <0.52, <0.54, <0.56, <0.58, <0.60, <0.62, <0.64, <0.66, <0.68, <0.70, <0.72, <0.74, <0.76, <0.78, <0.80, <0.82, <0.84, <0.86, <0.88, <0.90, <0.92, <0.94, <0.96, <0.98, <1.00.

n-CAP, nano-sized carbonate apatite; SrCl₂, Strontium chloride.

<sup>ab</sup>The same letter indicates no significant difference at α = 0.05 by Tukey’s multiple comparison One-way ANOVA test.

All values are the mean ± s.d.
large surface area. Therefore, they can have a high affinity and easily deposit on irregular spaces. In this study, these n-CAP were first applied to desensitizing dentifrice. Therefore, it can be expected that n-CAP would have a desensitizing effect by adhering to the dentinal tubules with strong affinity.

The methodology used in this study to evaluate the desensitizing dentifrices was different from other studies. A previous study evaluated only the SEM images which categorized the number of closing or patent dentinal tubules (3, 7). However, the new methodology of this study was to combine an image analysis system and SEM for a precise quantitative assessment of the dentinal tubule occluding effects. This study attempted to quantify the opening tubules area based on every SEM image of all samples using the image analyser.

The second different point was the main focus on the short-term effects of the dentifrices. A previous study only evaluated the long-term effects of desensitizing dentifrices such as 1000–50000 strokes (3). On the assumption that people generally brush three times per

**Fig. 1.** Scanning electron microscope images (magnified ×3000) of each group after tooth-brushing for 50 strokes: (a) etched dentin surface, (b) Non-n-CAP, (c) 5% n-CAP, (d) 10% n-CAP, (e) 20% n-CAP and (f) 10% SrCl₂. n-CAP is nano-sized carbonate apatite.

**Fig. 2.** Scanning electron microscope images (magnified ×50000) of the dentinal tubules after tooth-brushing for 50 strokes: (a) Non-n-CAP group, (b) 20% n-CAP group and (c) 10% SrCl₂ group. n-CAP is nano-sized carbonate apatite.
day after a meal, 10 strokes each (16); these strokes indicated a period of use for approximately 1–55 months, which meant fairly long-term use. According to the clinical relevance, an evaluation of the short-term effect of a desensitizing dentifrice will be more meaningful. Therefore, this study examined the effects of the short-term use from a minimum of 50 strokes to a maximum of 500 strokes. In particular, the tubule closing effect for 50 strokes by the desensitizing dentifrices will reflect the immediate self-recognition of pain relief for patients.

In terms of this result, the 20% n-CAP group showed the most effective occlusion of dentinal tubules when brushed for 50 strokes ($P < 0.05$). This group showed an occlusion rate of the dentinal tubules of 79.5% compared with the baseline. The 10% SrCl$_2$ group of the positive control also showed better tubular occlusion for 50 strokes than the other strokes. These results are in accordance with West’s study (7) in that the 10% SrCl$_2$ dentifrice group had a more closing effect during 1 min brushing than longer times. Table 1 indicated that there were closer relationships between the brushing frequency and concentrations of n-CAP. In the case of the 5% and 10% n-CAP, the groups showed the most occlusion effect of the dentinal tubule when brushed for 500 strokes. On the other hand, the 20% n-CAP group showed the highest closing effect during the short-term brushing for 50 strokes. In a clinical point of view, it will be a very useful tool for patients if the hypersensitive symptoms could be decreased within 2 days. The dentin specimen treated with 20% n-CAP was covered by minute nano-sized particles (Fig. 2b). Although these layers appeared to be simply covered and easily detachable, they were able to withstand ultrasonic cleaning for 30 min which was the pretreatment for SEM. Therefore, the n-CAP particles might strongly combine with the smear layer of the dentin surface rather than make a simple covering.

In terms of 500 strokes brushing, which indicated 17-day use, the 5, 10 and 20% n-CAP groups showed significantly higher occlusion effects than the SrCl$_2$ group ($P < 0.05$, Table 1). The n-CAP groups showed a 52–57% occluding rate. These results were also confirmed by SEM in which the groups containing n-CAP showed higher occlusion effects of the dentinal tubules than the SrCl$_2$ group. On the other hand, the SrCl$_2$ group showed similar aspects in the baseline specimen, which showed complete opening of the dentinal tubules (Fig. 3d). This can be explained by the fact that the SrCl$_2$ particles had been removed by continuous brushing for 500 strokes. Although the main mechanism of the SrCl$_2$ dentifrice, which is a well-known desensitizing dentifrice, has been reported, there was no distinct dentin tubule occlusion observed in this study after brushing for 500 strokes. A previous study also failed to demonstrate a direct tubule occlusion effect by the SrCl$_2$.
dentifrice after 4-week use (17). Another study reported the possible mechanism of the decreasing effect of dentin hypersensitivity by SrCl$_2$ and considered the interruption of neural transmission rather than a direct tubule closing effect (4). Therefore, our result of SrCl$_2$ is in accordance with previous research.

However, n-CAP which are more similar to the inorganic component of teeth can easily adhere to the smear layer produced by brushing. All the n-CAP groups in this study contained 8% silica as abrasives, which might also affect the increasing factors of dentinal tubule closing (7). Therefore, this combination with n-CAP and silica might have a synergistic effect on dentinal tubule closing. On the other hand, the content of abrasives, such as silica can affect the abrasivity of the dentifrice. The silica concentration used in this study was relatively low compared with other commercial products, which range from 20% to 40% (18).

Although the abrasivity of the n-CAP dentifrice was not tested in this study, a previous study (19) reported that the relative dentin abrasivity of a dentifrice containing 30% n-CAP without silica (relative dentin abrasivity (RDA)-3) showed much lower abrasivity than the British Standards Institute reference dentifrice (RDA-100). Hence, the dentifrice containing 20% n-CAP and 8% silica might have low abrasivity.

There were some limitations in this study. Our in vitro model only evaluated the outcome of continuous brushing, which does not reflect a real intermittent brushing situation. Therefore, there was possibility of overestimating the dentinal closing effect compared with a real situation. Accordingly, further study will be needed using an oral simulation model to simulate an in vivo situation to anticipate the clinical situation. In addition, more study will be needed to evaluate the sustaining effect of n-CAP particles covering the dentine surface.

In conclusion, the dentifrices containing n-CAP were effective in occluding the dentinal tubules in short-term use such as tooth-brushing for 50 strokes and less. Particularly, the dentifrice containing 20% n-CAP showed the most effective occlusion of dentinal tubules for 50 strokes.

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