Effectiveness of nano-carbonate apatite in occluding dentinal tubules using oral simulation model

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Abstract

The aim of this study was to evaluate the effect of a dentifrice containing nano-carbonate apatite (n-CAp) on the occlusion of dentinal tubules using a pH cycling. Eighty human dentin specimens were divided randomly into the following four groups; the negative control group (G1: 0% n-CAp), experimental group (G2: 20% n-CAp), positive control groups (G3: Sensodyne®, G4: DenShield®). They were applied to a pH cycling model for 12 days. The experimental group containing 20% n-CAp showed an occluding rate of the dentinal tubule of 78% compare with the baseline and showed significantly difference occluding effect among the other groups ($p<0.05$). The dentifrice containing 20% n-CAp was the most effective in occluding the dentinal tubules.

Keywords: *Dentin hypersensitivity, Nano- carbonate apatite, Occluding effect, pH cycling*

1. Introduction

Dentin hypersensitivity, a common dental problem, is a short and sharp pain caused by stimuli to exposed dentin without any dental defect or pathology [1]. This distinctive pain is understood to be caused by the movement of tubule fluid through patent tubules [2]. Since the hydrodynamic theory was proposed by Brännström, several studies have reported that dentin hypersensitivity is caused by open dentinal tubules. Therefore, the theory is the leading assumption to explain the mechanism of dentin hypersensitivity [3-5]. Hypersensitivity occurs due to the removal of the enamel or cementum by parafunctional habits, acid erosion and defective restoration [6]. The main additional cause of dentin exposure is gingival recession, periodontal disease or improper toothbrushing [7]. Dentin hypersensitivity treatment is also theoretically based on the mechanism, and the treatment mechanism is divided as follows: reducing the excitability of the nerve fibers within the pulp and occluding the open dentinal tubules [8]. The occluding of dentinal tubules is accepted as an effective method in lowering permeability of dentin, reducing the movement of dentinal fluid and alleviating pain [9]. In particular, the move of fluid occurring in column-shaped dentinal tubules follows the Poiseuille-Hagen equation [10]. The diameter of dentinal tubule affects the fluid movement much because when the diameter increases 2 times, fluid movement increases 16 times. Therefore, since small changes in the tubule diameter give a large impact on fluid movement, occluding the dentinal tubule is an important strategy [11].

Most opinion of clinicians is that dentin hypersensitivity is frequent, but that it is not easy to control [12]. Since it is rare to control the dentin hypersensitivity with one single treatment method, most clinicians agree that stepwise and comprehensive treatment is required. It is necessary to remove predisposing factor initially and advance to other treatments [1]. Among the existing methods to control the hypersensitivity, the daily use of desensitizing dentifrice as a self-control method is a relatively easy, non-invasive and economical method, so it is widely recommended as a first line treatment [9][13]. Desensitizing dentifrices include active ingredients such as a strontium chloride, calcium carbonate, silica, sodium citrate, and sodium fluoride, all of which are effective in occlusion of dentinal tubules [14][15]. Strontium chloride (SC) was proven by a large body of clinical research and has been a representative desensitizing ingredient [1]. Also more biocompatible and effective ingredients such as calcium sodium phosphosilicate, hydroxyapatite and carbonate apatite have been actively researched [16][17]. Carbonate apatite, which is a sort of calcium phosphate, is closer to biologic apatite that exists in dental and osseous tissues than hydroxyapatite, which is widely used in the medical and dental fields. Therefore, it is expected to be highly biocompatible [18]. Particularly, nano-sized carbonate apatite (n-CAp) that was developed recently creates a protective layer in the dentin. A previous study reported that this component has superior occluding effect of dentinal tubules compared with dentifrice containing SC [19]. The study evaluated the occluding effect of dentinal tubules through tooth brushing for 50, 100, 250 and 500 back and forth strokes. However, these results did not reflect the dynamic intra-oral situation as follows: first, there was no exposure to the acid environment that can reopen dentinal tubules. Second, saliva, which is able to cause a variety of modifications to the protective layer created by toothbrushing with dentifrice, was excluded. Therefore, the aim of this study is to evaluate the effect of the dentifrice containing n-CAp on the occlusion of dentinal tubules using a model that combines toothbrushing with a pH cycling process to reflect a real intra-oral situation.

2. Materials and Methods

2.1. Preparation of dentine specimens

Dentin specimens used in this study were obtained from recently extracted human molars without caries. The teeth were sectioned horizontally below the cemento-enamel junction using a diamond wheel disc. The root portion of tooth was first embedded with an acrylic resin into a teflon mold (19 x 12 x 8 mm). Each dentin specimen was wet ground using silicon carbide paper (600~2000 grits) on a polishing machine (RB 209 Minipol; R&B Inc, Seoul, Korea) to expose a flat dentin surface. In order to minimize of the inter-sample difference in each group, one dentin specimen was cut in five parts (2 x 2 x 2 mm) and again embedded with vinyl poly siloxane into a five-hole acrylic mold to get the dentin specimen without damage. Dentin specimens were treated with 6% citric acid for 1 minute to completely open the dentinal tubules (Figure 1).

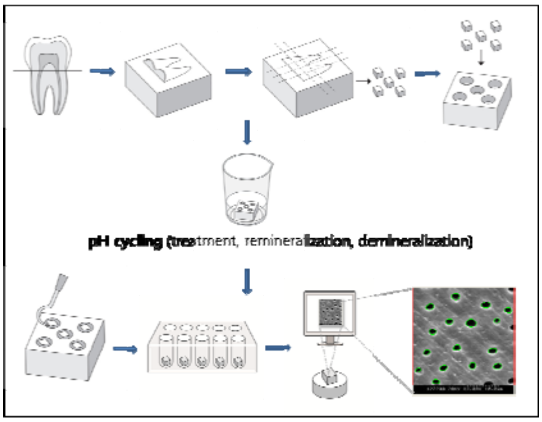

Figure 1. Schematic illustration showing experimental procedure

2.2. Experimental design

Eighty complete specimens were randomly classified evenly into four groups. In this study, the experimental dentifrice containing n-CAp and the negative control dentifrice that excludes only n-CAp and has the same other ingredients were evaluated. The positive control groups were assigned the dentifrice containing SC (Sensodyne Original®, GlaxoSmith Kline, U. S. A) and the dentifrice with calcium sodium phosphosilicate (NovaMin®) (DenShield™, NovaMin Technology, Inc., Alachua, U. S. A). All dentifrices used in this study exclude fluoride. All effective ingredients and abrasive agents are presented in Table 1.

Table 1. Ingredients of dentifrices used in this study

Groups	***N***	***Active* ingredients**	***Abrasive***
Negative Control	20	0% n-CAp	silica
Experimental	20	20% n-CAp	silica
Positive Control (1)	20	10% SC	calcium carbonate
Positive Control (2)	20	7.5% NovaMin	amorphous silica

2.3. De/remineralizing solution

Dentifrice slurries were prepared each time (1 dentifrice: 3 distilled water) and remineralizing solution used in the pH cycling has mixed artificial saliva with human stimulus saliva (1:1). Artificial saliva was made of gastric mucin (0.22%), KCl (0.114%), KH_2PO_4 (0.0738), NaCl (0.0381%) and $CaCl_2.H_2O$ (0.0213%) in 1000 ml distilled water and was controlled at pH 6.8. For demineralization solution, 50% calcium phosphate saturated 0.1M lactic acid was adjusted to pH 5.0 with the use of NaOH and HCl solution. The filtrate solution was mixed with 0.5% Cabopol (ETD 2050, Noveon Inc., USA) and was adjusted to pH 5.0 with the NaOH.

2.4. Experimental procedure

All specimens were applied to a chemical pH cycling model for 12 days to reappear a real oral situation. Toothbrushing was performed for 1 minute 3 times a day with a brushing machine (V-8 Cross Brushing Machine, Sabri, USA). The force of the toothbrush on the specimens was standardized as 150 g, and toothbrushing was performed 50 times per min. To simulate the oral pH demineralization time during the intake of food, the specimens were immersed in demineralizing solution for 4 hours a day, followed by immersion in the remineralizing solution. After the treatment, all specimens were carefully removed from acrylic mold and were dried in oven at 60 ℃ for 24 hours.

2.5. Dentinal tubule size determination using SEM and image analyzer

The surfaces of all specimens were evaluated by scanning electron microscopy (SEM) (S-800 Hitachi Ltd., Tokyo, Japan) to observe the size changes in dentinal tubules. At baseline the dentinal tubule was open completely after acid treatment. All the specimens in each group were evaluated randomly from the three portions of the specimens after pH cycling for 3, 6 and 12 days. The occluding effect of dentinal tubules was quantified using an image analyzer (Image-Pro PLUS v6.0, Media Cybernetics, Silver Spring, MD, USA). Unlike several studies in which the number of opened dentinal tubules was counted or randomly measured [20], this study measured the area of all dentinal tubules in each SEM image and evaluated the mean value of each group as in Lee' study [19] .

2.6. Statistical analysis

The means of the tubular area between the groups were analyzed by one-way ANOVA and Tukey's post hoc analysis using the SPSS 12.0 statistical package program (SPSS V12.0K, SPSS Inc., Chicago, Illinois, USA).

3. Results

After pH cycling for 12 days, the size of the dentinal tubules had decreased in all groups. Before tooth brushing, the mean values of the dentinal tubule area in the etched baseline specimens ranged from 5.84 to 6.33 μm^2 in each group. However, after cycling for 3 days, the area of the dentinal tubules in all groups had decreased greatly compared to the baseline. The experimental group (20% n-CAp group) and the positive control groups (10% SC group & 7.5% NovaMin group) showed statistically significant higher values than the negative control group in terms of dentinal tubules occluding effect ($p<0.05$). In addition, the result of the 6 days treatment indicated that dentinal tubule area of the 20% n-CAp group decreased 73% compared to baseline, and that there was a statistically significant difference between the experimental group and the negative control group without n-CAp ($p<0.05$). However, in the case of the treatment for 12 days, the area of dentinal tubules increased again in all groups except for the experimental group. 20% n-CAp showed an occlusion rate of the dentinal tubule of 78% compare with the baseline and showed significantly higher occlusion effects than the negative control group and the positive control groups ($p<0.05$). The 10% SC group showed 70% less open tubular areas than the baseline during the treatment for 3 days. Therefore, this group had the highest level of tubule occlusion during the initial time (Table 2). The areas of the dentinal tubules in most groups had decreased rapidly at the initial cycling, and dentinal tubules were slowly opening with the passing of time (Table 2). However, the tubule area of 20% n-CAp group had decreased continually during the treatment.

Table 2. Comparisons of dentinal tubule area after cycling for 12 days (unit: μm^2)

Groups	***N***	***Treatment* periods**			
		baseline	***3* days**	***6* days**	***12* days**
0% n-CAp	5	6.22 ± 1.23^{a}	3.79 ± 0.45^{a}	2.69 ± 0.35^{a}	3.16 ± 1.02^{a}
20% n-CAp	5	6.33 ± 0.53^{a}	2.62 ± 0.54^{b}	1.72 ± 0.44^{b}	1.38 ± 0.40^{b}
10% SC	5	5.98 ± 0.59^{a}	1.80 ± 0.53^{b}	2.65 ± 0.74^{ab}	3.05 ± 0.93^{a}
7.5% NovaMin	5	5.84 ± 0.90^{a}	2.21 ± 0.63^{b}	2.05 ± 0.54^{ab}	3.04 ± 1.14^{a}

All values mean the Mean ± Standard Deviation
[a,b,c] The same letter indicates no significant difference at $\alpha=0.05$ by Tukey's multiple comparison test.

Figure 2 shows the change of tubules size through the SEM images after pH cycling for 3 days and 12 days. The baseline specimen that was etched with 6% citric acid for 60 sec showed a higher number and a widening of the dentinal tubules. On the other hand, after cycling for 3 days, most of dentinal tubules in the 20% n-CAp group (b), 10% SC group (c) and 7.5% NovaMin group (d) were occluded and fewer in number. Particularly, in the case of 7.5% NovaMin group, big particles that are presumed to be calcium sodium phosphosilicate compound were observed on the surface of the specimen (Figure 2-d). However, the negative control group without desensitizing component was occluded partially by a smear layer from tooth brushing but overall, this group was similar to the baseline image (Figure 2-a). After the 12 days cycling, the small particles that covered the specimen surface in most groups were removed slowly and dentinal tubules had opened again. In contrast, the specimen surface of 20% n-CAp group was combined with minute particles and continued the occluding effect of dentinal tubules (Figure 2-b). When the dentinal tubules were observed with a high magnification SEM in cross sectional view, the tubular orifices in positive control groups were occluded with a thin layer or some dentinal tubules were opened. However, the tubular orifices of the 20% n-CAp group were occluded mostly by the thick layer (approximately 4.4 μm) (Figure 3).

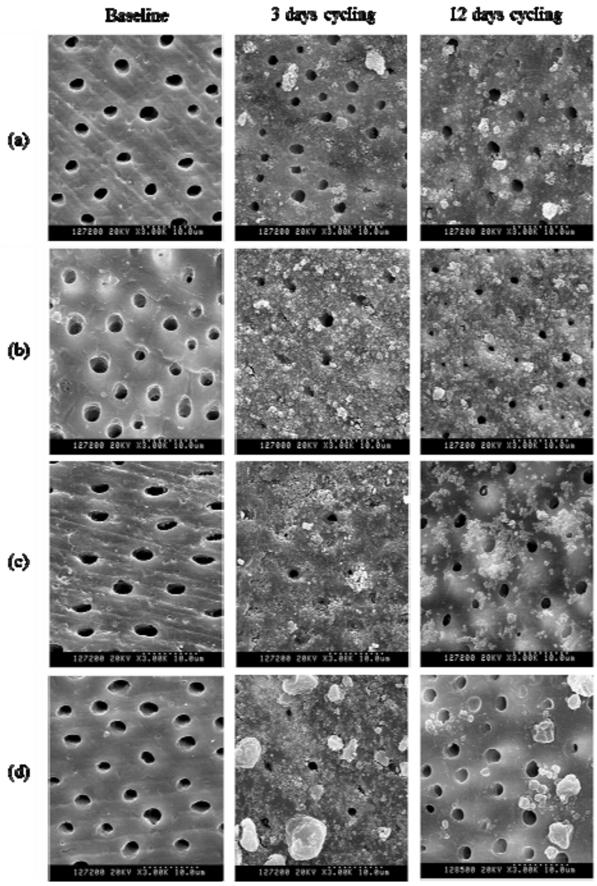

Figure 2. Fe-SEM images (x 3000) of dentinal tubules area after cycling for 3 days and 12 days: (a) 0% n-CAp; (b) 20% n-CAp; (c) 10% SC; (d) 7.5% NovaMin

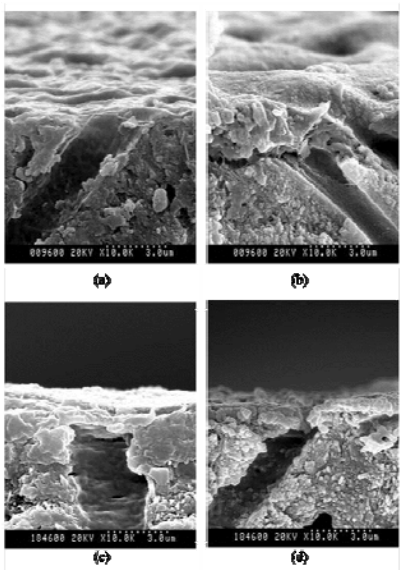

Figure 3. . Cross-sectional images (Fe-SEM, x 10000) of dentinal tubules after cycling for 12 days: (a) 0% n-CAp; (b) 20% n-CAp; (c) 10% SC; (d) 7.5% NovaMin

4. Discussion

The pH cycling was originally designed to simulate dynamic variation of environmental factors related to dental caries through the repeated performance of demineralization and remineralization [21]. This study used pH cycling, which is widely used for in vitro remineralization research, to simulate acid attack and the immersion caused by saliva that affects the occluding effect of dentinal tubules and the maintenance of a created protective layer in the actual oral environment. For observation of changes caused by repeated toothbrushing, five specimens of each group were picked on the 3 days, 6 days, and 12 days of the cycling to be evaluated by the SEM and the image analyzer.

After treatment for 3 days, the dentinal tubule area of all groups greatly decreased. Even the 0% n-CAp group, which did not include effective ingredients, the dentinal tubule area decreased and lasted continuously compared with baseline. That seems to be caused by silica, an abrasive agent included in dentifrice. It is known that the abrasive agent itself is attached to dentin or is combined with dentine smear debris to be deposited on the dentin surface and thereby to present the occluding effect of dentinal tubule [22]. The 20% n-CAp, the 10% SC and the 7.5% NovaMin had significantly lower dentinal tubules areas than the 0% n-CAp group. The SC is combined with phosphor in dentinal tubules and is substituted with calcium of hydroxyapatite in the dentinal tubule wall to create strontium phosphate crystal and thereby reduce the dentinal tubule permeability [23], and it is reported that it has a 75.5% desensitizing effect [24]. Dentin tubular area of the 10% SC group reduces greatly in the initial treatment. These result matches well with the study of West [25]; the study reported that the dentifrice containing 10% SC was the highest on occluding effect of dentinal tubule in the case of a short-time toothbrushing (1 min).

On the other hand, the NovaMin, a bioactive glass, was originally developed for alternative bone substitute with bone formation capability [26]. However, it is reported recently that it has the occluding effects on the dentinal tubule by being deposited on the dentin surface [27-29]. Its capability to control dentin hypersensitivity as an effective ingredient is proposed. A previous study demonstrated that dentifrice containing NovaMin showed 35% decreased pain of air stimulus, 39% reduced pain of cold water in a clinical trial performed for 6 week [16]. These results indicated that the dentifrice containing the NovaMin was more effective in reducing dentin hypersensitivity than the dentifrice containing SC, which shows 11% and 22% decreased pain, respectively to air stimulus and cold water. The mechanism of the Bioglass on occluding of dentinal tubules is understood to occur when the Bioglass release sodium and react with calcium and phosphate to create hydroxyl carbonate apatite when exposed to body fluids such as saliva [30]. In this study, because dentinal tubules are exposed enough to saliva through treatment, the occluding effect of the NovaMin is considered to be maximized.

After cycling for 12 days, all groups except for the 20% n-CAp group were reopened dentinal tubules. The dental tubule area of 20% n-CAp group decreased 78% compared to baseline, and was significantly different between the negative control group and positive control groups ($p<0.05$). The SC and the NovaMin were slowly removed from the surfaces of dentin by the physical stimulus of toothbrushing over time (Fig. 2). However, the n-CAp that is more similar to dental mineral component than hydroxyapatite and whose surface area is larger than the hydroxyapatite, was more strongly attached to the smear layer caused by toothbrushing, so the occluding effect of dentinal tubule continued. A previous study reported that n- CAp was able to adsorb on the tooth surface well because it has high surface energy [31].

Also, this study observed the cross-sectional surface of the specimen to identify the thickness of the deposition layer. The tubule orifices of most specimens were opened or clogged partially by a thin layer. However, in the case of the 20% n-CAp group, the entrance of dentinal tubules was occluded by a thick layer. This result can explain the fact that the n-CAp layer was used as a source to occlude the entrance of the dentinal tubules [32].

In summary, the dentifrice with 20% n-CAp, the occluding effect of dentinal tubule was maintained over long term use as well as short term, and thus the 20% n-CAp dentifrice is considered to be effective in reducing dentin hypersensitivity. However, this study reflected oral situations indirectly, yet are still in-vitro study. Therefore, further studies require clinical trial on patients with hypersensitivity symptoms.

5. References

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