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Remineralization Potential of New Toothpaste Containing Nano-Hydroxyapatite

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Abstract. Hydroxyapatite is the main component of enamel that gives the tooth a bright white appearance and eliminates the diffused reflection of light by filling up the fine pores of the tooth surface. Accordingly, remineralization of the teeth can be expected to some extent if hydroxyapatite is used to treat an incipient caries lesion (early white spot lesion). In addition, the remineralization effect will be increased if the particle size of hydroxyapatite can be reduced to less than that of the micron-size in existing toothpaste preparations. The aim of this in vitro study was to evaluate the effect of nano-hydroxyapatite toothpaste, which was produced by nano-technology, on the remineralization of human enamel. A tooth specimen, on which artificial incipient caries had been induced, was immersed into two toothpaste slurries for remineralization. One contains nano-sized hydroxyapatite and fluoride, and the other contains nano-sized hydroxyapatite excluding fluoride. In order to evaluate the remineralization effect, the Vickers Hardness Number & SEM image of the enamel surface was evaluated at each step. There were significant differences in VHN values between those obtained before and after the remineralization steps. The results showed that the remineralization effect increased with increasing immersing time ($P<0.05$). However, there were no significant differences in VHN values between the two groups ($P>0.05$). SEM also demonstrated differences the in micro surface at each step. In conclusion, a toothpaste containing nano-sized hydroxyapatite has the potential to remineralize an incipient caries lesion. In addition, the addition of fluoride had no synergistic effect on remineralization.

Introduction

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a major component of the hard tissue in the human body. Recently, tooth whitening and remineralization by HA toothpaste has attracted a great deal of attention particularly in dentifrice field [1]. Hydroxyapatite is the main component of enamel, which gives the tooth its bright white appearance and eliminates the diffused reflection of light by filling up the fine pores of the tooth surface. In addition, HA acts as an abrasive in toothpastes and removes the discoloration of the tooth surface. Therefore, remineralization of teeth can be expected to some extent if hydroxyapatite is used to treat an incipient caries lesion (early white spot lesion). Moreover, the effect of remineralization is expected to be more pronounced if the particle size of hydroxyapatite can be reduced to less than that of the micron-size in current toothpaste preparations.

Recently, nanotechnology has attracted a great deal of attention. Nanotechnology comprises technological developments on the nanometer scale, usually 0.1 to 100nm. There are many clinical effects of nano sized HA in the dental field. This nanotechnology makes the HA particle finer, which means that a remineralization effect of a demineralized tooth surface can be expected.
The aim of this in vitro study was to evaluate the effect of Nano-Hydroxyapatite toothpaste on the remineralization of human enamel.

Materials & Method

Erupted permanent teeth were cleaned by storing them in distilled water for at least 24 hours after their root and pulp had been removed. The proximal side was sectioned with a diamond wheel disk and enamel pieces were embedded in an epoxy resin for the fixation of the tooth surface. The enamel samples were then smoothed and polished to mirror flatness on a lapidary wheel using wet abrasive sandpaper up to 1000 grit for the VHN determination and SEM evaluation [2].
The baseline VHN was measured prior to demineralization. A total of 22 tooth specimens were assigned to 2 groups by matching the VHN stratification.
The demineralization solution used to induce the artificial incipient caries lesion comprised of lactic acid (0.1M), calcium ions (15mM) and phosphate ions (9mM). The solution was made from in 90% lactic acid ($CH_3CH(OH)COOH$, M.W. 90.08, Specific gravity 1.21), calcium chloride powder ($CaCl_2$· 2H2O, M.W. 147.03) and potassium phosphate powder (KH_2PO4, M.W. 136.09). The pH was then titrated to pH 4.3 using sodium hydroxide with constant monitoring with a pH meter. Calcium and phosphate ion were added to minimize the loss of the tooth surface and to induce subsurface enamel incipient caries during demineralization. It is known that a pH of pH 4.3 can induce artificial incipient caries [3]. The enamel samples were immersed in the demineralization solution at room temperature with constant stirring (200 rpm) for 12 hours. Subsequently, the enamel samples were carefully washed using tap water to remove any excess acid. The VHN of tooth specimen was then measured.
The toothpaste slurries for remineralization were prepared by mixing 100 grams of toothpaste in 100 ml distilled water. One group (Group A) contained nano-sized hydroxyapatite and fluoride (Sodium monofluorophosphate 0.65%), and the other (Group B) contains nano-sized hydroxyapatite excluded fluoride. Because fluoride strongly affects the remineralization of demineralized teeth, toothpastes containing nano-sized Hydroxyapatite excluding fluoride were used as the control.
Remineralization was carried out at room temperature in such way that the remineralizing solution (200ml) was continuously stirred (200 rpm) and the enamel sample remained static [4]. The deposition of nano-sized hydroxyapatite on the demineralized enamel surface was induced by immersing the specimen in the toothpaste slurries for 24 (remineralization Ⅰ) or 48 (remineralization Ⅱ) hours. After remineralization, the enamel samples were carefully washed in tap water and dried prior to analysis.
In order to evaluate the remineralization effect, the VHN of the enamel surface was measured after the remineralization Ⅰ or Ⅱ step using a micro hardness tester (JT TOSHI INC, Japan) [5,6]. Three indentations were made on each sample before and after each treatment using a single load (1000gf) function. The load function consisted of a linear loading segment, a holding segment (10s) at the peak load, and a linear unloading segment. The VHN at the adjacent place was measured at every step in order to minimize the error of hardness because the surface micro hardness could be measured variably on only one tooth. The VHN derived from the three indentations on each sample was averaged.
Scanning Electron Microscope (SEM) was also used to examine the micro surface of the specimens of each steps.
The results obtained on the micro hardness determination between each step were analyzed using repeated measure ANOVA and a multiple test. The differences between each group were compared using a t-test. Statistical analysis was performed using the Window SAS 8.2 statistical package (SAS Institute, Inc. U.S.A).

Results

Table 1 shows changes in the VHN values at each step according to demineralization and remineralization in each group.

The micro hardness of the enamel surface increased after the demineralized tooth specimens had been immersed into the toothpaste slurry containing the nano-sized hydroxyapatite,. There were significant differences in the VHN values before and after the remineralization steps ($P<0.05$). The remineralization effect increased with increasing immersing time. However, the VHN values were similar in groups A and B at all stages ($P>0.05$).

Table 1. VHN values (Mean ± SD) of each steps

Group	Baseline	Demineralization (12 hours)	Remineralization Ⅰ (24 hours)	Remineralizartion Ⅱ (48 hours)
A	326.8 ± 25.5	293.2 ± 18.1	298.8 ± 19.7	316.7 ± 14.1
B	322.7 ± 16.0	293.4 ± 16.3	312.3 ± 14.2	315.4 ± 14.1

SEM demonstrated that there were observable differences in micro surface at each step. Fig. 1 shows SEM images highlighting the change in the microstructure of the tooth surface at each step by SEM. Mineral loss was observed after demineralization and an intact enamel surface was observed after treating with the toothpaste slurry containing the nano-HA for remineralization.

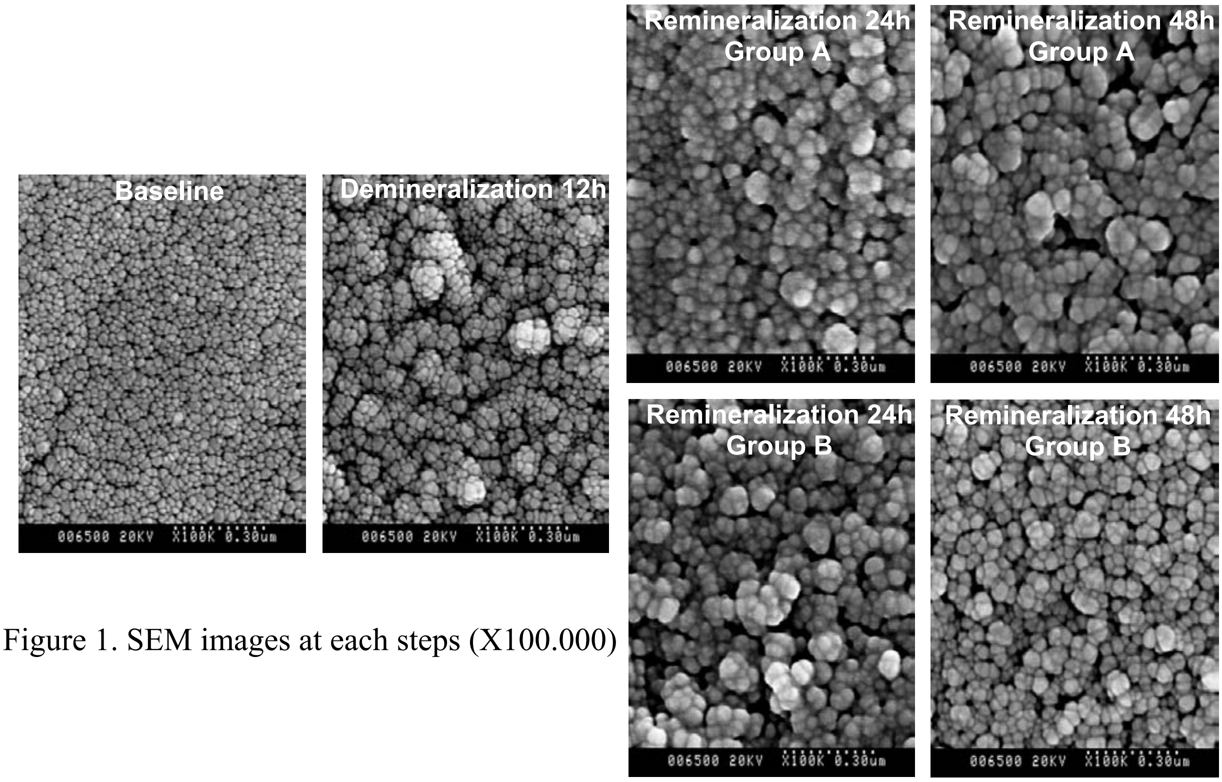

Figure 1. SEM images at each steps (X100.000)

Discussion

These results suggested that nano-sized hydroxyapatite toothpaste might have a beneficial effect on the remineralization of incipient dental caries. It is possible that nano-sized hydroxyapatite particles can fill the fine pores of the demineralized enamel surfaces. There were some limitations to this study. Toothpaste containing micron sized HA was not used as control group in this study. In addition, de-remineralization of the teeth does not occur continuously under normal conditions as

was shown in this study but intermittently. Therefore, an additional study will be needed to compare toothpaste containing micro sized HA with toothpaste containing nano-sized HA and to develop a delicate in vitro pH cycling de-remineralization model.
In this study, remineralization was also induced by deposition on a demineralized tooth in a tooth slurry containing nano HA. Therefore, an oral rinse solution containing HA might be used to prevent caries.
There was no synergistic effect of fluoride on remineralization in this vitro study. It was expected that the remineralization effect would be more pronounced when the fluoride ions of the toothpaste interact with calcium and phosphate ions in saliva [7]. Therefore, a future study will evaluate the synergic effect of fluoride by adding calcium and phosphate ions into the tooth slurry.

Conclusions

A toothpaste containing nano-sized hydroxyapatite has the potential to remineralize an incipient caries lesion. In addition, the addition of fluoride had no synergistic effect on remineralization.

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