Effect of Polyols on Phosphorus-Containing Calcium Fluoride Deposition on Hydroxyapatite Surfaces

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Abstract: The present study investigated how polyols (sugar alcohols) affect the formation of calcium fluoride (CaF2) in the presence of fluoride on synthetic hydroxyapatite (HA) as a model of tooth enamel. HA plates were immersed in sugar alcohol solutions containing 10-60 (w/w)% xylitol, D-sorbitol, or glycerol and 1000 ppm fluoride at room temperature to analyze CaF2 formation on the surfaces. Only glycerol enhanced fluoride incorporation significantly and induced a nanoscale spherical deposition on the HA surface composed of calcium, fluoride, and a small amount of phosphorous according to energy-dispersive X-ray spectroscopic analysis. Overall results suggest that glycerol is capable of dissolving HA surfaces and enhancing phosphorous-containing CaF2 deposition, which can potentially prevent dental caries through the enhanced remineralization of enamel surfaces. Thus, fluoride may be involved in controlling the entire process of CaF2 deposition, dissolution, and remineralization of enamel.

Key words: Calcium fluoride, Enamel, Fluoride, Hydroxyapatite, Polyols

Introduction

Hydroxyapatite (Ca10(PO4)6(OH)2, HA) is a commonly considered prototype for inorganic mineral components of teeth and bones1-5. The equilibrium between demineralization and remineralization maintains a sound tooth surface in the oral environment6. Demineralization is the process of HA dissolution and release of calcium (Ca2+) and phosphate (PO43-) ions from teeth, while remineralization is the nucleation of HA or other calcium phosphate crystals6. Streptococcus mutans and other microorganisms produce organic acids and cause a decrease in pH around the tooth surface, which shifts this equilibrium towards demineralization because the HA surface is susceptible to acidic pH. Repetition of this phenomenon frequently leads to dental caries formation. Changes in the visual characteristics of enamel (white spots/lesions) are found in the initial stages of caries formation. There are some studies showing that the use of fluoride (F-) containing products promote remineralization of teeth, inhibiting the progression of caries and eliminating the appearance of white spots/lesions4,7,8. The effect of F- on caries prevention is reportedly on enamel surfaces requires 100 and 300 ppm of F- in pH 5 and neutral solutions, respectively9,10,11.

There are many reports about caries prevention using F- containing toothpastes. The preventive effect of toothpastes containing 1500 ppm F- has been shown to be superior to those with 1000 ppm F-12. Nordström et al. reported that 5000 ppm F- toothpaste was significantly effective in the prevention of caries compared to that containing 1450 ppm F- after 2 years of use13. Therefore, development of toothpastes that successfully prevent dental caries requires higher F- concentrations and/or increased uptake of F- into the enamel. However, from a safety viewpoint, including large amounts of F- in oral care products for daily use is not recommended.

Polyols, such as glycerol, xylitol and sorbitol have been used as humectants or sweeteners in toothpaste14,15. Among them, xylitol and sorbitol have been studied in terms of their effects on caries prevention and remineralization16,17. These polyols molecules form a complex with Ca2+18,19, facilitating Ca2+ transport toward enamel surfaces. If applied directly to enamel surfaces, these polyols not only enhance remineralization, but also suppress dissolution of Ca2+ and PO43-, which slows advancement of demineralization20. Previous studies have also shown that remineralization is both further enhanced if xylitol is used with F-21,22. The mechanism by which this occurs is thought to be an increase in the driving force behind formation of fluoridated HA23. However, how these polyols are involved in enhancing the mineralization in the presence of F- is not fully understood in relation to the nature of apatite surfaces. In the polyols used in the toothpaste, glycerol has been investigated as a solvent for biomaterial synthesis24. However, there seems to be no report so far regarding the effect of glycerol on the mineralization and dissolution.

The aim of the present study was to develop caries prevention techniques using polyols with a constant concentration of F- and investigate how they affect the deposition of CaF2 onto model HA enamel surfaces in the presence of F-. The present results show the distinct effect of sugar alcohols on enhancement of CaF2 formation on HA surfaces by incorporating F- from the surrounding solution, which could explain the anticaries effect on enamel surfaces.

Materials and Methods

Uptake of F- on HA

HA plates (10 × 10 × 2 mm3) were purchased from Hoya Corp. (Tokyo, Japan). HA plates were smoothed by polishing with wet abrasive paper (P1500, Sankyo-Rikagaku Co., Saitama, Japan), then immersed in 10 ml
of various concentrations [10-60 (w/w)%] of polyol(xylitol, D-sorbitol and glycerol) solutions containing 1000 ppm sodium fluoride (NaF) at 25°C for 3 min. After, HA plates were washed with deionized water and dried naturally in air. Then, the HA plates were immersed in 1 M HCl for 30 s. The F concentration of the solution was measured by an Orion 9609BN F electrode (Thermo Electron Corp., Waltham, MA, USA). Each experiment was replicated four times and F uptake amounts on HA plates were calculated in μg/cm².

**Electron microscopy**

The HA plates after their immersion were sectioned with a low-speed diamond saw (Isomet 2000, Buehler, Lake Bluff, IL, USA) to obtain 3-mm thick disks. The disks were polished (smoothed) with wet abrasive paper (A3-1 SHT; 3M Co., Tokyo, Japan) and then immersed in 5 ml of 40 (w/w)% aqueous glycerol solution containing 1000 ppm NaF or aqueous NaF solution (1000 or 5000 ppm) for 30 min. After washing with deionized water and drying in air, the surface morphology of deposits on disks was observed by field emission scanning electron microscope (EM) (JSM-6330F, JEOL, Tokyo, Japan) with a platinum coating. To observe longitudinal sections of deposits, disk specimens were prepared using an FB-2100 focused ion beam (Hitachi High-Technologies Co., Tokyo, Japan). The surface of the HA disk samples was treated with carbon and tungsten to protect them from gallium ion beam damage during focused ion beam processing. Processing was performed at 40 keV with a beam current of 60 nA and 70 pA final thinning steps. Prepared thin sections were observed by scanning transmission electron microscope (STEM; HD-2300, Hitachi High Technologies Co., Tokyo, Japan). The composition of a selected area was obtained by an energy dispersive X-ray spectroscope (EDS; GENESIS 4000, Ametec Co., Tokyo, Japan).

**Results**

**Uptake of F on HA**

It was shown that the amount of F uptake on HA plates treated with polyol solutions containing 1000 ppm F (Fig. 1). When the plates
were treated with 1000 and 5000 ppm F\(^{-}\) alone, the average ± standard deviation F\(^{-}\) uptake was 0.23 ± 0.08 and 2.29 ± 0.07 μg/cm\(^2\), respectively. The amount of F\(^{-}\) uptake on HA plates treated with xylitol or D-sorbitol solutions was mostly constant regardless of the concentration of sugar alcohol. However, the amount of F\(^{-}\) uptake on HA plates increased with increasing glycerol concentration up to 50 (w/w)%, indicating glycerol improved F\(^{-}\) uptake on HA.

**EM observation of deposits on HA**

It was shown that field emission scanning EM images of untreated HA plates, those treated with 1000 and 5000 ppm F\(^{-}\) only, and plates submersed in 40 (w/w)% glycerol and 1000 ppm F\(^{-}\) (Fig. 2). The results showed that 1000 ppm F\(^{-}\) induced a rough surface on initially smooth HA plates. Treatment with 5000 ppm F\(^{-}\) alone and 1000 ppm F\(^{-}\) with glycerol not only caused rough surface topography, but also growth of small precipitates on the surface of HA plates. Although treatment with 5000 ppm F\(^{-}\) alone induced growth of cuboidal precipitates (100-500 nm), treatment with 1000 ppm F\(^{-}\) and glycerol generated spherical precipitates (75 nm).

It was shown that STEM images of HA plates treated with 5000 ppm F\(^{-}\) and 40 (w/w)% glycerol (Fig. 3). The platinum used for vapor deposition covered the surface of the cuboidal precipitates on 5000 ppm F\(^{-}\) treated HA plates (Fig. 3a). On the other hand, the platinum had invaded inner parts of the spherical precipitates on the glycerol and 1000 ppm F\(^{-}\) treated HA. These results suggest that the precipitates in each condition have different inner structures, as well as shapes.

It was shown that the STEM images and EDS profiles of cross-sections of HA plates treated with 5000 ppm F\(^{-}\) and those treated with 40
shape and solubility created in the presence of 5000 ppm F precipitated in glycerol exhibited a spherical morphology, whereas those profiles, which suggests deposition of CaF spherical crystals enriched with Ca the HA plate in glycerol containing 1000 ppm F have been enough to accelerate CaF analysis revealed that more than 300 ppm F formation of CaF with xylitol and D-sorbitol. Larsen et al. have shown that spontaneous increased in the presence of more than 20% glycerol compared to uptake onto HA surfaces. We revealed that the amount of F materials, like on teeth.

Different types of F uptake have been shown to occur on the surface of enamel: firmly-bound and loosely-bound F (as CaF$_2$) can work as a reservoir of F in enamel and support remineralization of firmly-bound F because CaF$_2$ shows higher solubility and provides F at a neutral or lower pH. Recently, it has been revealed that CaF$_2$ nanoparticles are also effective at suppressing formation of Streptococcus mutans biofilms. However, since a high concentration of F in topical agents presents potential health risks, our current study focused on sugar alcohols as toothpaste additives to promote uptake of F onto the apatite materials, like on teeth.

The present results demonstrated that among various polyols, only glycerol can promote F uptake and allow CaF$_2$ crystals to precipitate onto HA surfaces. We revealed that the amount of F uptake on HA increased in the presence of more than 20% glycerol compared to uptake with xylitol and D-sorbitol. Larsen et al. have shown that spontaneous formation of CaF$_2$ requires an F ion activity product attained from a specific F concentration in saturated neutral solution with respect to enamel apatite crystals in the presence of Ca and PO$_4^{3-}$ ions. Their analysis revealed that more than 300 ppm F can promote precipitation of CaF$_2$. Thus, treatment with 1000 ppm F in the current study should have been enough to accelerate CaF$_2$ precipitation. When we immersed the HA plate in glycerol containing 1000 ppm F, we observed many spherical crystals enriched with Ca$^{2+}$ and F from STEM and EDS profiles, which suggests deposition of CaF$_2$. Interestingly, CaF$_2$ crystals precipitated in glycerol exhibited a spherical morphology, whereas those created in the presence of 5000 ppm F were cuboidal. The different crystal structure is produced by the environment of growing crystals; solvent choice and crystallization conditions are known to affect crystal shape and solubility.

Table 1. Elemental analysis of regions of interest (Points 1-6) on scanning transmission electron microscopy images (Figure 4).

<table>
<thead>
<tr>
<th>Element</th>
<th>Deposits 5000 ppm F</th>
<th>Glycerol + 1000 ppm F</th>
<th>HA plate 5000 ppm F</th>
<th>Glycerol + 1000 ppm F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Point 1</td>
<td>Point 2</td>
<td>Point 3</td>
<td>Point 4</td>
</tr>
<tr>
<td>Ca</td>
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<td>17.7</td>
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<tr>
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<td>50.3</td>
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</tr>
<tr>
<td>Na</td>
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<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
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</tr>
</tbody>
</table>

Discussion

F treatment is very effective for caries prevention in enamel. Two different types of F uptake have been shown to occur on the surface of enamel: firmly-bound and loosely-bound F (as CaF$_2$). CaF$_2$ can work as a reservoir of F in enamel and support remineralization of firmly-bound F because CaF$_2$ shows higher solubility and provides F at a neutral or lower pH. Recently, it has been revealed that CaF$_2$ nanoparticles are also effective at suppressing formation of Streptococcus mutans biofilms. However, since a high concentration of F in topical agents presents potential health risks, our current study focused on sugar alcohols as toothpaste additives to promote uptake of F onto the apatite materials, like on teeth.

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Present results showed that glycerol not only induced promotion of CaF$_2$ formation on HA, but also changes in crystal shapes, suggesting that glycerol affects CaF$_2$ particle formation. Efficient formation of CaF$_2$-like materials (loosely-bound F) on enamel is thought to be important for more effective promotion of remineralization by fluoridating agents because they can release an effective concentration of F under mild acidic conditions in the mouth. Previously, Christoffersen et al. reported that CaF$_2$-incorporated phosphorous crystals were roughly spherical. However, in our EDS analysis, phosphorous was detected in both square (5000 ppm F) and spherical (glycerol plus 1000 ppm F) crystals. Although the detailed mechanism is still unclear, glycerol adsorbed onto CaF$_2$ may affect the shape of precipitates. Our findings suggest that combination of glycerol and F has the potential to promote CaF$_2$ precipitation on the enamel surface. Further study is necessary to investigate the effect of glycerol and F for clinical applications. Nonetheless, we expect that CaF$_2$ generated from glycerol treatment as a supplier of F has the potential to contribute to caries prevention.

In conclusion, the present study confirmed that glycerol is capable of inducing F deposition on synthetic HA surfaces. We revealed that glycerol was more effective than xylitol and D-sorbitol at enhancing uptake of F on HA surfaces. This is the first study showing that glycerol works not only as a simple humectant in toothpastes, but also as a material for controlling F uptake on the enamel surface.

Acknowledgements

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Conflict of interest

The present study was carried out in part by a Joint Research program with the Kao Corporation.

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