Assessment of Vickers hardness, microstructure, and surface roughness of dentin after initial dissolution by acidulated phosphate-fluoride

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Abstract  Acidulated phosphate-fluoride (APF) is widely used for the prevention of enamel caries. The topical application of fluoride is a promising treatment for improving the acid-resistance of tooth, promoting its remineralization, and improving its crystallinity. APF contains a high concentration of fluorine ions (9,000 ppm); it first decalcifies the apatite in dentin, generating calcium and phosphate ions, which bind to fluoride ions, thus precipitating calcium fluoride. APF is prepared as an acidic solution (pH3.5) and is used to reinforce the enamel. However, dentin is less resistant to acid than enamel. As such, APF treatment at the same acidity carries a risk of weakening the dentin after application. In many cases, wedge-shaped defect (WSD), root caries, and attrition can cause dentin exposure, to which the APF can attach. Therefore, in this study, we analyzed the effect of APF treatment on dentin by measuring Vickers hardness, making observations with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), and measuring average surface roughness (Ra). The findings of this study suggested that an acidulated phosphate fluoride (APF) treatment of 3 min or longer caused marked dissolution of dentin and posed the risk of deterioration of its initial strength. Therefore, careful management is required to preclude dentin dissolution when performing APF treatment for preventing dental caries.

Key words  Acidulated phosphate fluoride, Demineralized, Dentin erosion

Introduction

In recent years, there has been a marked decrease in dental caries due to the development of prevention methods, oral hygiene management in pediatric dentistry, and patients’ growing interest in preventing dental caries. The use of fluoride and lasers is indicated in the prevention of dental caries, with topical application of fluoride being widely used and extremely effective. Topical application of fluoride can improve the acid-resistance of dentin, promote its remineralization, and improve its crystallinity. The method for the topical application of fluoride was devised by Bibby, and Cheyne in the 1940s; Knutson et al. later proposed applying sodium fluoride solution. In the 1950s, Howell et al. proposed the use of stannous fluoride solutions, and in the 1960s, Brudevold et al. proposed the use of acid phosphate-fluoride (APF) solutions. These topical fluoride application methods are still commonly used today. These methods have many benefits, including formation of fluoroapatite, improvement of the crystallinity of the enamel, promotion of remineralization, and antimicrobial activity, and are thus considered an effective means of preventing dental caries. APF is prepared as an acidic solution with a high concentration of fluorine ions (9,000 ppm). Once applied to a tooth, it initially decalcifies the surface. Calcium ions and phosphate ions are then generated; calcium ions bind to fluoride ions, and this leads to the precipitation of calcium...
Some fluorine ions act on hydroxyapatite to generate hydrofluoroapatite and fluoroapatite. Moreover, it is thought that precipitated calcium fluoride is gradually dissolved by the saliva to improve the crystallinity and inhibit the solubility of the hydroxyapatite that composes the enamel, and increase the acid-resistance of the enamel. In short, APF is an acidic preparation used for precipitating calcium fluoride. APF is meant for enamel treatment and is quite effective in improving acid-resistance. APF treatment of dentin has been well studied; Vale et al. have reported that the acid resistance of dentin was improved by APF treatment, and Saunders et al. have elucidated that root dentin was effectively protected by 1.23% APF gels. In addition, studies have suggested that APF treatment might have an inhibiting effect on hypersensitive dentin. However, dentin is less resistant to acid than enamel, and therefore when treated with the same level of acidity, carries a risk of dissolving and thus weakening the dentin. In this study, we analyzed the effect of APF treatment on dentin by measuring Vickers hardness, making observations with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM), and measuring the surface roughness of arithmetic mean (Ra).

Materials and Methods

Specimen preparation

Bovine dentin was used for the experiments. Frozen bovine teeth were thawed at room temperature. Caries, cracks, and coloration were investigated using a stereoscopic microscope, and teeth that could interfere with the experimental results were excluded. The selected bovine teeth were washed with a physiological saline solution; dental plaque, tartar, and deposits were removed using a scaler and a Robinson brush, and ultrasonic cleaning was performed for 30 minutes. In addition, all elements of the washed bovine teeth, except for the dentin, were completely removed by cutting with an air turbine. The teeth were then sliced perpendicularly to their long axis with a low-speed hard-tissue cutting machine (Isomet Bhuller, Germany). Once cut, the dentin fragments were embedded in cylinders (10 mm in diameter and 8 mm in height) using an embedding mold and a self-curing acrylic resin. To ensure that an area of approximately 5 mm × 5 mm of fresh dentin was exposed, the teeth were polished under running water using waterproof abrasive paper (#400 to #2000, Carborundum waterproof paper, RIKEN, Japan) and were mirror-polished using 9-μm and 3-μm wrapping films. After ultrasonic cleaning for 30 minutes, these were used as the samples. To reduce the influence of individual differences between the teeth, 12 teeth were used, with each tooth divided into 7 equal parts, which were then sorted into separate experimental groups. Samples for the series of experiments were stored in hermetically sealed containers at 100% relative humidity.

APF treatment of the specimens

The exposed dentin was blown with air and was then treated with an APF solution (Furoden A, 9,000 ppm F pH 3.5, Sunstar, Japan). After the surface moisture was completely removed with filter paper, the APF solution was absorbed with 0.05 g of absorbent cotton and applied to the dentin. The specimens were then left to rest. The specimens were categorized as untreated or APF-treated for 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, and 10 minutes. After treatment, the samples were washed with running distilled water for 60 seconds.

SEM observation of the dentin surface

The untreated and APF-treated specimens were observed by SEM. Carbon sputtering of the specimens was performed using a carbon coater, and the specimens were observed at low and high magnification using an SEM.

Measurement of Vickers hardness after APF treatment

The Vickers hardness of the dentin surface was measured after APF treatment. The measurements were performed using a Vickers microhardness tester at 100 g for 5 seconds. Vickers hardness was calculated based on the diagonal length of the formed indentation.

CLSM observation and Ra measurements

After APF treatment, the bird-view of the dentin surface was observed by CLSM, and the surface roughness of arithmetic mean (Ra) was measured. The dust on the dentin surface was removed by an airstream. Using utility wax (GC, Japan), the specimens were fixed on micro-glass slides perpendicularly to the observation axis. The observations were performed using a violet laser with an excitation
wavelength of 408 nm and an observation range of $1,350 \times 1,012 \mu m$. After laser scanning, three-dimensional images of the specimens were constructed using reconstruction software (VK Analyzer KEYENCE), and top-down views of the surfaces were observed. The Ra of the specimens was measured using the same reconstruction software.

**Statistical analysis**

A one-way analysis of variance (ANOVA) was performed for Vickers hardness and Ra using SPSS for Windows 9.0J advance model. When significant differences were found, multiple comparisons were performed using Scheffe’s method ($n = 5$).

**Results**

**Observations by SEM**

Figure 1 shows a SEM image of the untreated and each time APF treated dentin after polishing. Spherical apatite crystals of less than 1 nm composing the dentin and polishing marks of about 1 nm were visible at the surface of APF untreated. The surface exhibited a slightly uneven structure. The surface roughness of the specimens treated for 1 minute was slightly smoother than that of the untreated specimens, and the polishing marks also appeared slightly shallower and smoother. In addition, nearly uniform spherical particles of several nm in diameter were present on one side of the surfaces. The surfaces of specimens treated for 2–3 minutes were smoother than those treated for 1 minute. Spherical precipitates of various sizes, presumed to be calcium fluoride, were found at the surface. The surfaces of specimens treated for 4–10 minutes became increasingly smooth with increased duration of APF treatment. In addition, the diameters of the spherical precipitates were larger, and the precipitates were denser. Large polishing marks remained present for all durations of APF treatment.
but smaller polishing marks became shallower, smoother, and almost disappeared in images taken at higher magnification.

**Vickers hardness (HV)**

Figure 2 shows Vickers hardness and standard deviation of the dentin after APF treatment. After 0 minutes of APF treatment, the HV was 51.0 ± 2.0. After 1 minute of APF treatment, the HV significantly decreased to 40.7 ± 1.4, an approximately 20% weakening. The hardness was significantly reduced with treatments of 3 minutes or longer compared to the 2-minute treatment. However, no significant differences in HV were found between specimens treated with APF for 3–10 minutes. After a 10-minute APF treatment, the HV was 36.1 ± 2.2, representing an approximately 30% decrease in hardness compared to untreated specimens.

**CLSM images**

Figures 3 through 5 show CLSM images of the dentin surface after treatment. Polishing marks were clearly visible at the surface of untreated specimens. In addition, dentinal tubule openings were visible at various places. As in the SEM images, the surface smoothness increased and the polishing marks became thinner with longer APF treatment durations. In addition, the diameter of the dentinal tubules also showed a slight tendency to expansion.

**The surface roughness of arithmetic mean (Ra)**

Figure 6 shows Ra measurements and standard deviation of the APF-treated dentin surfaces. The Ra value 1 minute after treatment was significantly reduced compared to that of untreated specimens. The Ra after a 2-minute, 4-minute, 5-minute, and 10-minute treatment was significantly reduced compared to after a 1-minute treatment. However, there were no significant differences between the 2-minute treatment and longer treatment durations.

**Discussion**

**The response of dentin after APF treatment**

As shown in the following equation, high concentrations of fluorine on the enamel cause the dissolution
Fig. 4 Typical CLSM bird view of dentine surface after APF treatment (1–3 min)

Fig. 5 Typical CLSM bird view of dentine surface after APF treatment (4–10 min)
of hydroxyapatite and generate calcium fluoride$$^2$$.$$^{21}\) Ca_{10}(PO_4)_6(OH)_2 + 20F^-→ 10CaF_2 + 6(PO_4)^{3-} + 2(OH)^-$$

Calcium fluoride, which precipitates due to the APF treatment, was initially believed to be eluted in the saliva in a relatively short time$$^{22,23}\). However, results from subsequent studies have shown that, at low pH in the presence of HPO_4^{2-}, the surface of the calcium fluoride is insoluble; as a result, the dissolution rate decreases, and it may take 2 weeks or longer to elute the fluoride ions$$^{22,23}\).

The precipitated calcium fluoride acts as a reservoir of fluorine. In association with a decrease in pH inside the oral cavity due to organic acid production by dental plaque, the precipitated calcium fluoride slowly releases fluorine ions into the saliva$$^{24}\). Recent perspectives on fluorine’s protective effects against dental caries have focused on the coating effect of fluoride loosely bound to the periphery of the crystals, as well as on the effects of coexisting fluorine ions, rather than on stabilization through fluorine substitution in hydroxyapatite crystals (fluoroapatite creation)$$^{25}\). Brudevold et al.$$^9\) previously studied the effect of pH on APF function. They reported a marked uptake of fluoride ions by APF solutions at a pH of 3.0. Wellock et al.$$^{10}\) reported that this same APF formulation in schoolchildren protected against dental caries, and was significantly more effective compared to local application of sodium fluoride solutions. The amount of precipitated calcium fluoride is influenced by various factors, such as the pH of the solution, the type and concentration of fluoride, the type of acid, and the duration of its action$$^8,26\). In addition, the rate of dissolution of apatite depends on the composition of the solution; with changes in pH and the solution’s composition also occurring as the apatite dissolves$$^{22,27}\). Thus, the rate of apatite dissolution, pH, and the rate of calcium fluoride precipitation are interconnected factors that cannot be easily dissected. In this study, commercially available APF with a pH of 3.5 was used. The Vickers hardness was significantly reduced even after a 1-minute treatment, likely due to dissolution of the dentin. More neutral sodium fluoride solutions might be more suitable for achieving acid-resistance while maintaining dentin hardness immediately after treatment. However, at a neutral pH, the dentin is less likely to be calcium-ion-rich and could produce less calcium fluoride. Therefore, detailed studies should be performed on the effects of pH, concentration, fluoride properties, and duration of treatment on the dentin.

**Fluoride treatment of dentin in clinical settings**

In the field of pediatric dentistry, the topical application of fluoride is common. However, dentin may also be partially exposed due to attrition, abrasions, WSD, and root caries in the oral cavity. In particular, cases of dentin exposure at the occlusal surface and incisal edge due to dental attrition, as well as partial exposure of dentin due to hypoplasia, can be encountered in the field of pediatric dentistry. In our experiments using commercial APF with a pH of 3.5, Vickers hardness decreased by approximately 20% after a 1-minute treatment and by approximately 30% after a 10-minute treatment. This is likely a result of dentin demineralization due to the acidic pH of the APF preparation. There was a significant
decrease in Vickers hardness after 2 and 3 minutes of treatment, but 3- to 10-minute treatments did not result in significant changes. The mechanical strength of dentin has been studied on the basis of its mineral content\textsuperscript{28,29}, and a decrease in Vickers hardness indicates decreased mineral content\textsuperscript{30,31}. APF produces calcium fluoride while causing the demineralization of hydroxyapatite in the dentin, and therefore, APF is prepared to have an acidic pH of about 3.5. However, the demineralizing effect is stronger in dentin than in enamel and results in a decrease in Vickers hardness. Even a 1-min APF treatment has been indicated to cause a significant decrease in mineral content. In addition, similar findings were observed with Ra, which decreased after only a few minutes of treatment. The acidity of APF causes dissolution of the dental surface, i.e., smoothening of the roughness of the surface. Our experiments showed significant differences between the 1-min treatment and the 2-, 4-, 5-, and 10-min treatments; however, no significant difference was found between 1-min treatment and 3-min treatment. Although the cause remains unknown, we believe that individual differences in the mineral density of the specimens subjected to 3-min treatments and the directions of the dentinal tubules therein may have contributed to the abovementioned findings. Consistent with the decrease in Vickers hardness, the Ra values also showed a tendency to decrease in 1-min treatments. Our findings thus indicate that even a 1-minute treatment can cause marked dissolution of the dentin, suggesting a considerable risk for weakening the dentin. The actual clinical impact of a 10% decrease in dentin hardness is not yet clear. The advantage of APF is that it improves the acid-resistance of the treated region. Although frequent APF treatment of exposed dentin over a short period of time could potentially improve acid resistance through the formation of calcium fluoride, a further weakening of the tooth structure could occur immediately after application. Therefore, careful management will be required to preclude dentin dissolution when performing APF treatment for preventing dental caries. In addition, the use of neutral fluoride, which is less likely to cause dentin weakening, needs to be considered.

**Conclusion**

Here, we studied the effects of APF on Vickers hardness, surface structure, and Ra of dentin. The results show that even a 1-minute treatment caused a significant decrease in Ra and Vickers hardness, likely due to dissolution of the dentin, and the same treatment was also found to cause a slight expansion of the dentinal tubules. Therefore, when applying APF in the oral cavity, it is important to apply it selectively to the enamel and not the dentin.

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