Distribution of elements in teeth and inhibition of demineralization by titanium fluoride: Effects of concentration and pH in a titanium fluoride solution

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The purpose of this study was to assess the effects of titanium fluoride (TiF₄) concentration and pH on fluoride distribution and demineralization of root dentin surfaces. Concentrations of 0.1%, 1%, and 2% TiF₄ (pH 1), 1% TiF₄ solution adjusted to pH 4, 5, 6, and 1.35% sodium fluoride (NaF) solution were applied to root dentin surfaces. Each specimen was subjected to pH cycling (pH: 4.5–7.0) for 4 weeks. Lesion depth and calcium, fluorine, and titanium distribution were then evaluated. Our limited study indicates that lesion depth and fluorine and titanium distribution in dentin depend on the concentration of a TiF₄ solution. We also found that a 1% TiF₄ solution adjusted to a pH 4–6 can reduce demineralization as effectively as a similar concentration of NaF.

Keywords: Titanium fluoride, Inhibition of demineralization, Element distribution, PIXE/PIGE analysis

INTRODUCTION

Fluoride application is widely recognized as an effective treatment that can prevent dental caries. Fluoride works by 1) increasing the resistance of tooth structure to demineralization, 2) enhancing remineralization, and 3) inhibiting bacterial metabolism. A variety of fluoride treatments have been applied to caries prevention. For self-application, low-dose/high-frequency (daily use) or high-potency/low-frequency (weekly use) mouth rinses and fluoride dentifrices are commonly used. Professional treatments include higher concentrations of acidulated phosphate fluoride gel and sodium fluoride (NaF) application to tooth surfaces.

Recently, fluoride combined with metal ion has reportedly proved to be more effective at inhibiting demineralization compared with fluoride use alone. Strontium5-8, stannous fluoride9,10, and zinc11-15 were demonstrated to be more effective against demineralization compared with NaF. Silver diamine fluoride can also contribute to caries prevention, while titanium fluoride (TiF₄) is reportedly more effective at reducing mineral loss and lesion depth compared with NaF.16,17 Nanohardness and the modulus of elasticity of dentin surfaces increased substantially after application of 2.5% TiF₄.16 One study found that a combination of β-cyclodextrin and TiF₄ exhibited a greater potential to inhibit demineralization of inner enamel17 and another reported that TiF₄ could induce a low level of apoptosis on human gingival fibroblasts.18 However, most of these studies used a high concentration of TiF₄ (4%, 3%, or 2.45%) and low pH values (1.0 or 2–3). Solutions of 2% TiF₄ and 2.7% NaF both contain 12,200 ppm of fluorine, a concentration that is greater than that of topical fluoride applications commonly used for weekly treatments (less than 900 ppm). Solutions with high concentrations of TiF₄ (2% and 4%) can decrease demineralization to a greater extent compared to low concentrations. However, such high-concentration solutions are not intended for patient use but rather should be applied by professionals. Low pH values may result in over-demineralization of tooth surfaces. For home use, fluoride products should be low in concentration and close to neutral in pH.

The aim of the present study was to evaluate the effects of TiF₄ concentration and pH on fluoride distribution and demineralization on root dentin surfaces to determine appropriate values for home use.

MATERIALS AND METHODS

Sample preparation

The use of extracted teeth in the present study was approved by the Research Ethics Committee of Asahi University School of Dentistry (Approval Number: 30008). The buccal root dentin surfaces of the extracted human molars were ground with SiC paper (grid #600) for preparation of flat surface. The tooth specimens were sliced perpendicularly along the long axis, and obtained from the area which extended to approximately 2 mm from the cement enamel junction to the root apex, using a low-speed diamond saw (Isomet; Bueeler, Lake Bluff, IL, USA) and polished with a grindstone (#2000) to produce 200 µm-thick slices (Fig.1). The sliced tooth specimens were coated with wax, with the exception of the exposed flat dentin surface (approx. 2-mm width), and subjected to an automatic pH cycling system (6 cycles/
day) for 4 weeks to prepare artificial carious dentin. A demineralizing solution (pH 4.5) containing 0.2 M lactic acid, 3.0 mM calcium chloride (CaCl₂), and 1.8 mM potassium dihydrogen phosphate (KH₂PO₄) was prepared along with a remineralizing solution (pH 7.0) of 0.02 M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 3.0 mM CaCl₂, 1.8 mM KH₂PO₄, and 130 mM potassium chloride. At 7:00 AM, 10:00 AM, 1:00 PM, 4:00 PM, 7:00 PM, and 10:00 PM, pH changes were induced by flowing the demineralization solution at a rate of 50 mL/min for 2 min. During the cycling periods, specimens were soaked weekly for 5 min in some kinds of TiF₄ solutions or a NaF solution (Table 1). These fluoride treatments were carried out at 11:30 AM every Tuesday. Each specimen was picked up and rinsed with deionized water (DW) for 2 min and then soaked with TiF₄ or NaF solution for 5 min. After that specimens were washed with DW for 10 min and then returned to pH cycling. Our research used TiF₄ solution of 0.1% TiF₄ (group 0.1T), 1% TiF₄ (1T), or 2% TiF₄ (2T) and 1% TiF₄ (pH 1) solution adjusted to pH 4, 5, or 6, (P4, P5, and P6, respectively) for TiF₄ treatment, and a 1.35% NaF solution (NaF) with a fluoride concentration equivalent to that of the 1% TiF₄ solution for fluoride treatment. Control (Cont) involved no fluoride application during the testing periods. Nine samples from each group were subjected to analysis.

**Measurement of lesion depth**

After pH cycling, wax coatings were removed with xylene. Each specimen was mounted on a glass plate and covered with a second, smaller glass plate with water, and then observed under a polarizing microscope (Nikon OPTIPHOTO; Nikon, Tokyo, Japan) at 20× magnification. The lesion depth of each specimen was measured with ImageJ software. The depths at three points of each specimen were measured and averaged.

**Measurement of fluorine, titanium, and calcium distribution**

After measurement of lesion depths, the fluorine, titanium and calcium contents of the specimens were measured using an in-air μ–particle-induced X-ray emission (PIXE)/particle-induced gamma emission (PIGE) system at the Takasaki Ion Accelerators for Advanced Radiation Application (Takasaki, Japan). The procedure for measurement of specimens followed those described in previous reports. Each specimen was attached directly to the window at the end of a 1.7-MeV proton beam apparatus and bombarded in ambient air conditions. The beam spot diameter was approximately 1 µm, and the beam current was 100 pA. Fluorine content was measured with PIGE by the nuclear reaction ¹⁹F(p,αγ)¹⁶O and gamma rays were detected with a 81 cm² sodium iodide detector 4 mm behind the sample. Titanium and calcium contents were measured by PIXE, and simultaneously detected with a silicon-lithium detector in a vacuum. The distance and the angle of the detector were 118 mm from the target and 40° to the beam axis, respectively. For quantitative analysis, beam intensity was monitored in terms of X-ray yield from a copper foil by switching the beam onto the foil for 3 s every 30 s (Fig. 2). Elemental concentrations were

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### Table 1 Fluoride solutions used in this study

| Group | Solution |
|-------|----------|
| 0.1T  | 0.1% TiF₄ |
| 1T    | 1% TiF₄ (pH 1) |
| 2T    | 2% TiF₄ |
| P4    | 1% TiF₄ adjusted to pH 4 |
| P5    | 1% TiF₄ adjusted to pH 5 |
| P6    | 1% TiF₄ adjusted to pH 6 |
| NaF   | 1.35% NaF (Fluoride concentration equivalent to that of the 1% TiF₄) |
| Cont  | No fluoride application |
measured in a sampling area 270 µm wide×500 µm deep ((M) in Fig. 1), with the data converted to a graphical resolution of 128×128 pixels. Quantitative results for fluorine and calcium were obtained as previously described. Titanium contents were measured as the ratio of titanium counts to copper counts due to a lack of titanium reference materials.

To compare fluorine and titanium distributions, the average concentration in each specimen was calculated at intervals of approximately 10 µm from a defined surface. The superficial surface of dentin was defined as the position at which the calcium concentration in intact dentin was 5%21,23). The cumulative fluorine concentration (ppm×µm) or titanium content (counts/copper counts×µm) in each specimen was calculated for an area 100 µm from the defined surface.

Statistical analysis
The obtained data were analyzed by a Kruskal-Wallis test and Steel-Dwass test (α=0.05). All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commandar that adds statistical functions frequently used in biostatistics24).

RESULTS

Lesion depth
Figure 3 provides representative polarized microscopy images for each group. The control group exhibited deeper demineralization compared with the other groups. Figure 4 shows the lesion depth of each group. All fluoride-treated groups exhibited smaller lesion depths compared with that of the control. In a comparison of TiF₄ solutions and NaF solution, the 1T and 2T groups displayed smaller depths than the NaF group. No other TiF₄ groups showed significant differences against the
With respect to concentration, the 1T and 2T groups displayed smaller depths than those of the 0.1T group, and for solution pH, the depths of the 1T group were smaller than those of the other pH groups. Meanwhile, there were no significant differences in lesion depths among the P4, P5, and P6 groups.

**Calcium, fluorine, and titanium distribution**

Figure 5 depicts representative calcium, fluorine, and titanium mapping images. Fluorine was distributed into the tooth structure, and considerable levels of fluorine were found in the 1T and 2T groups. NaF group indicated the similar fluorine distribution to 1T group. Titanium was deposited on the superficial surfaces of all titanium-treated specimens and diffused into the lesion bodies of specimens 1T, 2T, and P4. As compared with fluorine, there was less titanium distributed in the dentin. Ti deposits were not observed in the NaF and control groups.

Figure 6 depicts cumulative fluorine distribution from the superficial surface to a lesion depth of 100 µm. The 1T and 2T groups had higher fluorine levels than the 0.1T group. The 1T group showed significantly higher fluorine content compared with the P4 and P5 groups, but no significant difference was evident between the 1T and P6 groups. Only the 1T and 2T groups indicated no significant difference with the NaF group. The control
Figure 7 depicts cumulative titanium distribution from the superficial surface to a lesion depth of 100 µm. The 1T and 2T groups had higher titanium levels than the other groups. Overall, as the pH of the solution increased, the value of titanium decreased. All TiF₄-treated groups showed a higher value when compared with the NaF and control groups.

DISCUSSION

To determine appropriate TiF₄ concentrations and pH values for application of topical fluoride, the effects of concentration and pH on demineralization and distributions of fluorine and titanium on root dentin surface were assessed. When TiF₄ and NaF groups were compared, group 1T displayed reduced depths and higher fluorine and titanium distributions compared with the NaF group, which had the same concentration of fluoride. Group 2T showed results similar to those of group 1T against NaF. TiF₄ reacts with enamel, forming an acid-resistant surface coating of the dental enamel. The coating layer is a titanium dioxide glaze-like layer, which is created by the low-pH of TiF₄, and favors linking between the titanium and oxygen of the phosphate group. Büyükyilmaz et al. reported that TiF₄-containing materials produce fluorine- and titanium-rich layers on applied surfaces. Our study deals with dentin surface, while the previously mentioned reports concern enamel. But because between 75% and 80% of dentin is composed of hydroxyapatite, the enamel results may be applicable to dentin surfaces. Morphological studies of TiF₄ treatment using scanning electron microscopy (SEM) found that the deposits on the surface layer appeared to be 0.1-µm globular particles. Other SEM studies showed that the massive surface coating on the enamel appeared to be retained even after washing with KOH solution even though this coating did not have the typical globular appearance of CaF₂. Büyükyilmaz et al. reported that a layer with a thickness of approximately 20 µm had covered the dentin surface, as determined by confocal laser scanning microscopy. By X-ray diffraction analysis, the peak of titanium dioxide (TiO₂) and titanium hydrogen phosphate hydrate (Ti(HPO₄)₂·H₂O) were detected on hydroxyapatite treated with TiF₄.

Comar et al. reported that TiF₄ can provide higher fluoride deposition rates compared with NaF in bovine enamel (although fluoride concentrations are similar in each compound). The amount of fluoride release into water from TiF₄ varnish is reportedly greater than that from NaF, a finding that is supported by our results. One of the reasons for low lesion depth may be the high degree of fluoride distribution uptake. More fluoride is reportedly associated with NaF at pH 1.2 than from TiF₄ at pH 1.2. However, as we did not use a low-pH NaF solution, we could not confirm those findings. Our results suggest that a glaze-like layer or other titanium compounds may be more effective options for reducing demineralization and lesion depths compared with ensuring large amounts of fluoride uptake through fluoride application.

As revealed by PIXE/PIGE analysis, fluorine was found deep in dentin, while titanium was deposited only superficially. The atomic weight and size of titanium are higher than those of fluorine. As a result, fluorine can pass through narrower areas and spread more widely. Titanium deposition may create a glaze-like layer and inhibit demineralization. Bastings et al. reported that TiF₄ application produced a precipitate-like surface layer deposited on intertubular and intratubular dentin, occluding dentine tubule density. This layer appeared to contribute to low demineralization at the dentin surface.

With respect to TiF₄, the high-concentration and low-pH groups were associated with shallower lesion depth and higher fluoride distributions, although no significant difference was observed between pH 1 and 6 for fluorine distribution. Daily applications of a 0.5% TiF₄ solution was reported to significantly reduce enamel erosion in vitro compared with 1% or 0.1% solutions. Lesion depths in dentin surfaces treated with 2% TiF₄ were shallower than those treated with 1%, 3%, and 4% TiF₄ and 2.7% NaF. A low-pH NaF solution was associated with greater microhardness and fluoride uptake compared with neutral-pH NaF. Our findings show that a high concentration (1 or 2%) of the TiF₄ solution contained greater amounts of elements. High deposits of each element were therefore detected in dentin. Gonzalez-Cabezas et al. suggested that a low-pH NaF solution can directly cause catastrophic damage to the crystalline structure of most demineralized areas of advanced non-cavitated lesions, exponentially reducing the number of nucleation sites. When the pH of a fluoride solution drops below 3, free fluoride in the acidic solution exists mainly in the form of undissociated hydrofluoric acid, which can easily penetrate the hydroxyapatite lattice. A 2% TiF₄ solution is reportedly associated with a certain degree of mineral dissolution, increasing surface porosity and promoting deeper penetration of calcium, phosphate, and fluoride. The same mechanism, much titanium was able to penetrate dentin using a low-pH TiF₄ solution. Our limited study reveals that lesion depth may be determined more by the amount of fluorine distribution than the amount of titanium, given TiF₄ solutions of similar concentrations. However, a lengthier experiment may produce different fluoride distributions or lesion depths among TiF₄ solutions of various pH values. Groups P4, P5, and P6 displayed lesion depths similar to those of the NaF group, but were associated with lower fluoride distributions. This suggests that TiF₄ may be reducing the demineralization rate compared with NaF and therefore decreasing mineralization can be expected with groups P4, P5, or P6. A pH of 6 is greater than the critical pH of demineralization, and group P6 showed no significant ability of demineralization and fluoride distribution compared with groups P4 and P5. A TiF₄ solution with a pH of 6 may effectively demineralize at the same degree as at a pH of 4 or 5, suggesting that pH...
6 is appropriate for the task. Salomão et al.\textsuperscript{30} reported that TiF\textsubscript{4} and NaF have similar cytotoxic effects on fibroblast viability. Use of a TiF\textsubscript{4} solution would therefore be effective in caries prevention. The effects of a concentration between 0.1% to 1% should be considered in more detail to determine appropriate concentrations and pH values for TiF\textsubscript{4} solutions when applied to caries prevention.

CONCLUSION

Our limited study indicates that the lesion depth in dentin surfaces and fluoride and titanium distribution in dentin are dependent on TiF\textsubscript{4} concentration, and a 1% TiF\textsubscript{4} solution adjusted to a pH of 4–6 can reduce demineralization comparable to that of an NaF solution at a similar concentration. The use of TiF\textsubscript{4} solutions at pH 4–6 is a superior treatment for caries prevention.

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