Introduction
In recent years, the number of remaining teeth in the elderly has increased due to prolongation of mean life expectancy and increased awareness of oral hygiene\(^1\). With the increase in the number of remaining teeth, physiological or pathological gingival recession is found in most dentate elderly individuals, aged 65 years and above, and the dentin in the tooth root area is exposed\(^2\). The increase in health-consciousness has led to changes in dietary habits, with increased intake of citrus fruits, while habitual intake of soft drinks containing citric acid, phosphoric acid, etc., has also increased. Thus, tooth erosion in a wide range of age groups, spanning from young to elderly individuals, is increasing markedly\(^3\).

Tooth erosion is defined as tooth dissolution due to chemical reactions that do not involve microorganisms\(^4\). Intrinsic and extrinsic factors cause tooth erosion. Intrinsic factors include chronic gastrointestinal disorders, while extrinsic factors include intake of citrus fruits, carbonated drinks, sports drinks, and alcohol, i.e., habitually ingested acidic foods\(^5\). In addition, tooth erosion has also been reported to occur in individuals engaged in occupations requiring the handling of strong acids, such as hydrochloric acid and sulphuric acid at battery producing factories. As such, occupational exposure is also a causal factor\(^6\). Among these causes, habitual ingestion of readily available acidic foods and drinks contributes most to the recent increase in tooth erosion.

Citrus fruits, carbonated drinks, and acidic beverages, such as soft drinks, contain high levels of erosive acids, such as lactic acid, phosphoric acid, acetic acid, and citric acid. In general, lactic acid is abundant in lactic acid bacteria-containing beverages; phosphoric acid is found in many carbonated drinks, acetic acid is found in the health drinks, and citric acid is found in citrus fruits. Moreover, some of these items, such as carbonated drinks, also contain sugars, increasing the risk of caries development due to their low pH and high titratable acidity\(^7\). When acidic food and drinks with a pH lower than the critical pH 5.5 of enamel come into contact with enamel, rapid demineralization occurs with loss of minerals such as calcium and phosphor, which are structural components of hydroxyapatite (HAp)\(^8\). Moreover, it has been reported that intake of acidic beverages causes a decrease in enamel hardness\(^9\). Dentin that is exposed due to gingival recession has a high collagen content, and its critical pH is also high at 6.0-6.2. It is therefore highly susceptible to acids\(^10\). Thus, dentin erosion is more rapid and extensive than enamel erosion. Therefore, early and effective action against tooth erosion is more necessary for dentin than for enamel. However, in current clinical practice in Japan, standard fluoride application is the only

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**Abstract:** In recent years, tooth erosion due to tooth root exposure has increased. This is associated with an increase in remaining teeth in the elderly and frequent ingestion of acidic foods. Fluoride application is a clinical method for preventing tooth erosion; however, dentin solubility after topical fluoride application, according to the type of erosive acid, has not been adequately investigated. We studied the characteristics of demineralization and acid resistance of dentin to four erosive acids and evaluated the efficacy of topical fluoride application in preventing tooth erosion. Small blocks with a smooth surface were prepared by mirror-finishing the labial side of a bovine tooth root. In the experimental group, acidulated phosphate fluoride (APF) was applied topically for 4 minutes, while the control group received no fluoride treatment. Both groups were immersed in a remineralization solution for 1 h at 37 °C. Five samples from each group were subjected to tooth erosion and demineralization by immersion in lactic acid, phosphoric acid, acetic acid, and citric acid for 6 h at 37 °C. Demineralization and dentin solubility were evaluated by calculating mineral loss (AZ) and lesion depth (Ld) from the average surface roughness (Sa), difference in height profile, and contact microradiography (CMR). We also performed electron probe microanalysis (EPMA) and X-ray photoelectron spectroscopy (XPS) qualitative analysis to assess fluoride dynamics and properties of the compounds formed on the dentin surface after APF application. Samples with APF application showed greater resistance towards all four erosive acids. While a highly calcified layer was found with lactic and acetic acid, resistance was not adequate against citric acid, which has chelating properties. Fluoridated calcium formed on the tooth surface after topical fluoride application. Fluoride ions are taken up by peritubular dentin through the dentinal tubules, resulting in increased acid resistance.

**Key words:** Acidulated phosphate fluoride, Contact microradiography, Dentin, Electron probe microanalysis, Tooth erosion
Acid challenge experiment

Half of the mirror-polished labial dentin, as well as the lingual surface and mesiodistal surface were coated with dental sticky wax. The samples were divided into two groups, an experimental surface that was fluoride treated (n = 20) and a non-F surface that was not fluoride treated (n = 20). In the experimental surface, the samples were placed in acidulated phosphate fluoride (9,000 ppmF, pH 3.6) for 4 minutes, as a topical fluoride application. The samples of the non-F surface were immersed (n = 20). In the experimental surface, the samples were placed in a demineralization solution used in this experiment was prepared such that the final concentration was Ca: 1 mM, P: 0.6 mM, F: 0.05 mM (100 ml), and each sample was immersed in 30 ml of the solution. After the remineralization treatment, five samples from each group were immersed in each erosive acid for 6 h at 37 °C to perform the tooth erosion and demineralization treatments. For the tooth erosion and demineralization treatment, lactic acid, phosphoric acid, acetic acid, and citric acid were each prepared to obtain a concentration of 0.1 M and pH 4.0. Samples were suspended in these acids in a 300 ml beaker, and each sample was made to react with approximately 50 ml of the demineralization solution. After the demineralization treatment, the samples were immersed in xylene to remove wax, and then, following the usual method, they were dehydrated using an ascending ethanol series and then subjected to the various measurements mentioned below. Due to the demineralization treatment, substance defects were seen on both the experimental surface and non-F surface and therefore, the height of the surface of the labial dentin that was covered with wax was set as the control surface; a virtual surface was also set. The height from the virtual surface to the base of the demineralization surface was set as the depth of the substance defect and was used to correct each type of measurement value.

3D measurement laser microscopic observation

Surface roughness and difference in profile height were measured using a 3D measurement laser microscope (LEXT OLS4000, Olympus Corporation, Tokyo, Japan). The calculated average surface roughness (Sa) was used to measure the surface roughness, and measurements were made as five sites for each sample, and the mean ± SD was determined. The measurement area was set at 645 × 645 µm, and the cut-off value was set at 80 µm. Similarly, to ascertain differences in the height profile, the position of the boundary of the control surface and demineralization-treated surface was measured at five sites, while avoiding thick dental tubules and contamination, and the mean ± SD was determined.

Contact microradiogram

The samples were embedded in polyester resin (Rigolac; Nisshin EM Co. Ltd., Tokyo, Japan) to prepare polished sections, 100 µm in thickness. Using a soft X-ray generator (Softex CMR-3; Softex Co., Ltd., Tokyo, Japan) equipped with a 20 µm thick Ni filter, imaging conditions for an aluminum step wedge with 20 steps, with each step of 20 µm, were set to enable differentiation from step 1 to step 20. Thus, imaging was performed with the tube voltage set at 15 kV, tube current at 3 mA, and radiation time of 5 min, and light microscopy was performed at 200 × magnification. For imaging, a glass plate (High Precision Photo Plate, HRP-SN-2; Konica Minolta Japan, INC. Tokyo, Japan) was used. The plate was placed in a developer (D-19: Kodak, Rochester, NY, USA) at 20 °C, to develop for 5 min. The plate was then fixed for 5 min and washed with water for 10 min. After drying, a cover glass was placed, and the plate was stored. Completed plate were converted to grayscale (8 bit, 256 tone) using image analysis software (Image Pro Plus, version 6.2; Media Cybernetics Inc., Silver Spring, MD, USA) and an image analysis system (HC-2500/OL; Olympus Corporation, Tokyo, Japan).
Electron probe microanalysis

EPMA observation was performed to examine fluoride distribution in dentin after the topical fluoride application, as well as the properties of the compounds formed after the treatment. The samples were prepared by treating them in APF solution (9,000 ppmF, pH 3.6) and then in remineralization solution for 1 h at 37 °C. They were then dehydrated using an ascending ethanol series. After carbon was evaporated from the samples, a qualitative analysis of the sample surface and analysis of the calcium, fluorine, and phosphorus surfaces were performed using EPMA (JXA-8200; JEOL Inc., Tokyo, Japan) and the concentration profile was acquired. The mineral loss value (ΔZ) and lesion depth (Ld) were measured and the extent of demineralization compared. Each of the five sites was measured in the range of 50 × 300 µm from the surface vicinity to the deep healthy dentin. For ΔZ, the mineral equivalent was calculated by the formula of Angmar et al., using the density of the sample and the aluminum step wedge that was captured at the same time as reference. The values were converted to a histogram with the mineral value taken as 0% and the healthy dentin section as 100%[12]. In addition, the depth of the demineralized layer (Ld) was obtained as the length from the original surface (i.e. before demineralization) to the area where the mineral content represents 95% of the healthy dentin[13].

X-ray photoelectron spectroscopy

The samples were treated in APF solution (9,000 ppmF, pH 3.6) for 4 min and then in the remineralization solution for 1 h at 37 °C. They were then dehydrated in an ascending ethanol series and polished to a thickness of 4 mm. Surface analysis was performed using an XPS analyzer (XPS, AXIS-ULTRA, Kratos Analytical, Manchester, UK) with the conditions of Al-Kα, 15 kV, and 10 mA. The spectrum of each element was obtained was corrected for hydrocarbon-binding energy, and each element was confirmed[13]. The conditions were acceleration voltage of 10 kV and irradiation current of 2 × 10-8 A.

Statistical analysis

The mean ± SD of five samples was determined for each measurement, and was presented as the mean ± SD of five replicates per four types of erosive acid. In the comparison of the four types of erosive acids, P-values were calculated by 1-way analysis of variance (ANOVA) and results were considered significant at p < 0.05. The Bonferroni test was used for post-hoc comparisons when significance was determined by ANOVA (p < 0.05). Graphs were prepared and data analyzed using a software (ORIGIN 2019b, Lightstone Corp, Tokyo, Japan).

Results

Comparison of surface roughness and difference in height profile after demineralization treatment

The Sa of the experimental surface and non-F surface treated with the various types of erosive acids are shown in Fig. 2. With lactic acid, the Sa of the experimental surface was 0.41 ± 0.02 µm and that of the non-F surface was 0.35 ± 0.04 µm, showing that the Sa of the experimental surface that was subjected to topical fluoride application was significantly large (p < 0.05) (Fig. 2). Similarly, with phosphoric acid, the Sa for the experimental surface was 0.36 ± 0.03 µm and that for the non-F surface was 0.24 ± 0.04 µm. With acetic acid, the Sa for the experimental surface was 0.53 ± 0.03 µm and that for the non-F surface was 0.31 ± 0.04 µm. With citric acid, the Sa for the experimental surface was 0.53 ± 0.05 µm and that for the non-F surface was 0.40 ± 0.12 µm. In all cases, the value for the experimental surface, which had been subjected to topical fluoride application, was significantly larger than that of the non-F surface (p < 0.05). Among the four types of acids, the difference between the experimental surface and non-F surface was largest with acetic acid, while it was the smallest with lactic acid. Comparison of the Sa in the non-F surface, which did not receive topical fluoride application, for the four acids revealed that citric acid produced the most roughness, followed by lactic acid and acetic acid, with phosphoric acid tending to produce the least roughness; however, there was no statistically significant difference among the four acids (ANOVA, p > 0.05) (Fig. 2). In the experimental surface that was subjected to topical fluoride application, Sa due to citric acid and acetic acid was large, while that due to phosphoric acid was the least, similar to the non-F surface (p < 0.05) (Fig. 2).

The difference in height profile between the experimental surface and the non-F surface with respect to the control surface after acid de- mineralization due to four types of acid erosion are shown in Fig. 3. Lactic acid caused a marked decrease in the difference in height profile of the experimental surface as compared to the non-F surface (Fig. 3A, B). The difference in height of the experimental surface was 15.49 ± 2.01 µm, which was a significant decrease as compared to 49.64 ± 6.13 µm for the non-F surface (p < 0.05, Fig. 4). With phosphoric acid, there was hardly any difference from the control surface in terms of the difference in height for both the non-F and experimental surface (Fig. 3C, D): 1.23 ± 0.97 µm for the experimental surface and 1.84 ± 0.74 µm for the non-F surface, showing no significant difference (p < 0.05; Fig. 4). For acetic acid and citric acid, the profiles are shown below. Compared to the difference in height of the non-F surface, that of the experimental
surface was decreased by about 1/2 to 1/3 with acetic acid and citric acid (Fig. 3E–H). With acetic acid, the value of the experimental surface was 12.40 ± 1.44 µm and that of the non-F surface was 37.43 ± 9.37 µm. With citric acid, the value of the experimental surface was 13.41 ± 2.67 µm and that of the non-F surface was 34.10 ± 16.08 µm (Fig. 4). With acetic acid and citric acid, there was significant difference between the experimental and non-F surface, and for both groups, the difference in height from the non-F surface was significant (p < 0.05).

Figure 3. Image of the demineralization boundary surface after treatment with erosive acids. The boundary of the control surface and the demineralization surface is shown. The left column represents the experimental surface (A, C, E, G) and the right column represents the non-F surface (B, D, F, H). The difference in height profile between the experimental surface and the non-F surface with respect to the control surface after acid decalcification due to four types of acid erosion are shown. There was a difference in height from the control surface in both experimental and non-F surface for lactic acid, acetic acid and citric acid. With phosphoric acid, there was hardly any difference from the control Surface in terms of the difference in height for both the non-F and experimental surface.
A comparison by acid type was also performed in the non-F group. Lactic acid showed the largest difference in height, followed by acetic acid and citric acid, while phosphoric acid showed hardly any difference (p < 0.05; Fig. 4). In the experimental surface, there was no significant difference among lactic acid, acetic acid, and citric acid. Only phosphoric acid showed a significantly smaller value than the other 3 acids (p < 0.05; Fig. 4).

Contact microradiography observation and image analysis

The CMR images of the boundary area of the experimental and non-F surface after the demineralization treatment with the four types of erosive acid are shown in Fig. 5. In the non-F surface, approximately 100 µm of substance defect due to demineralization was seen after demineralization treatment with lactic acid (Fig. 5B). The base of the demineralized surface was rough and part of the dentin surrounding the dentinal tubule remained intact. There was no image of demineralization from the low to the deep areas (Fig. 5B). For the experimental surface, the substance defect was approximately 5-10 µm, and a demineralization layer of uniform depth was formed approximately 50-70 µm from the surface layer. An image of a hypercalcified surface layer and typical demineralization below the surface, covering the demineralization layer, is shown in Fig. 5A. A value close to that of a non-demineralized area was seen in the deep layer, located approximately 100 µm from the surface layer (Fig. 5A).

With phosphoric acid, a distinct demineralization layer of the experimental surface could not be confirmed, and a value close to that of a non-demineralized area was obtained from the surface layer to the deep layer (Fig. 5C). In the case of the non-F surface, a partly deep demineralization layer was seen at the boundary with the control surface; however, there was hardly any substance defect and only slightly increased permeability in the region 20-30 µm from the surface layer (Fig. 5D).

With acetic acid, the substance defect on the experimental surface was only 5-10 µm and an almost uniform layer of demineralization formed, located at a depth of 120-130 µm from the surface. Only the surface layer was calcified, and in contrast to the typical demineraliza-
tion below the surface that covers the demineralization layer, hypercalcification was seen in a broad zone located 20-80 µm from the surface (Fig. 5E). In the non-F surface, a substance defect of approximately 100 µm of the flat bottom was seen (Fig. 5F).

With citric acid, a substance defect was seen in both the experimental surface and the non-F surface. A rough bottom of approximately 50 µm was seen in the experimental surface and of approximately 100 µm was seen in the non-F surface (Fig. 5G, H). In contrast to the other 3 types of acids, with citric acid, a hypercalcified layer was not seen even in the experimental surface; however, the substance defect of the experimental surface was the greatest among the four acids (Fig. 5G).

**Mineral loss value and lesion depth**

A graph comparing ΔZ after erosive acid demineralization, as analyzed by CMR imaging, is shown in Fig. 6. With lactic acid, ΔZ of the non-F surface was 15,915 ± 1,290 vol%·µm, while that of the experimental surface was 10,563 ± 1,382 vol%·µm, showing a significant decrease in the experimental surface (p < 0.05; Fig. 6). With phosphoric acid, the value for the non-F surface was 608 ± 279 vol%·µm, while that of the experimental surface was 131 ± 65 vol%·µm; there was no significant difference between the non-F surface and experimental surface (p > 0.05; Fig. 6). With acetic acid, the value for the non-F surface was 22,662 ± 2,791 vol%·µm, while that of the experimental surface was 5,095 ± 993 vol%·µm, which were statistically significantly different (p < 0.05; Fig. 6). Similarly, for citric acid, the value for the non-F surface was 24,800 ± 3,860 vol%·µm, while that for the experimental surface was 11,590 ± 1,047 vol%·µm, showing a significant decrease for the experimental surface (p < 0.05; Fig. 6).

When the amount of demineralization of the non-F surface was compared among the acid types, citric acid produced the highest value, followed by acetic acid, lactic acid, and phosphoric acid, in decreasing order. Compared to the other 3 acid types, the value was significantly smaller for phosphoric acid (p < 0.05). In the experimental surface, mineral loss was highest with citric acid and lactic acid, followed by acetic acid. Similarly, in the non-F surface, phosphoric acid showed the lowest result. Acetic acid produced the largest difference between the non-F surface and the experimental surface, as ΔZ of the experimental surface decreased up to approximately 1/4th of the value of the non-F surface.

Ld based on CMR image analysis is shown in Fig. 7. With lactic acid, Ld of the non-F surface was 127.99 ± 6.20 µm, while that of the experimental surface was 107.44 ± 6.18 vol%·µm, showing a significant decrease in the experimental surface (p < 0.05; Fig. 7). With phosphoric acid, the value for the non-F surface was 21.86 ± 7.15 µm, while that of the experimental surface was 21.67 ± 7.98 µm, which was not statistically significantly different between the non-F surface and experimental surface (p > 0.05; Fig. 7). With acetic acid, the value of the non-F surface was 21.86 ± 7.15 µm, while that of the experimental surface was 21.67 ± 7.98 µm, which was not statistically significantly different between the non-F surface and experimental surface (p > 0.05; Fig. 7). With acetic acid, the value of the non-F surface was 107.44 ± 6.18 vol%·µm, showing a significant decrease in the experimental surface (p < 0.05; Fig. 7). Similarly, with citric acid, the value of the non-F surface was 152.37 ± 14.46 µm, while that of the experimental surface was 119.02 ± 4.24 µm, showing a significant decrease in the experimental surface (p < 0.05; Fig. 7).

When the Ld of the non-F surface was compared for the various acid types, acetic acid produced the largest value, followed by citric acid, lactic acid, and phosphoric acid, in decreasing order. Compared to the other three acid types, phosphoric acid produced a significantly smaller value (p < 0.05). For the experimental surface, the Ld value was significantly higher for acetic acid, citric acid, and lactic acid as compared to that of phosphoric acid (p < 0.05; Fig. 7).

Figure 6. Comparison of mineral loss value (ΔZ) after erosive acid treatment. The black bars indicate the experimental surface and the white bars represent the non-F surface. The horizontal axis shows the various erosive acids. The longitudinal axis shows ΔZ values. For each sample, five sites from the vicinity of the surface to the healthy dentin in a region of 50 × 300 µm were measured. All five samples were measured and the mean ± SD is shown. For acetic acid, there was a significant difference between ΔZ on the experimental surface and ΔZ on the non-F surface.

Figure 7. Comparison of lesion depth (Ld) after treatment with the various erosive acids. The black bars show the experimental surface and the white bars indicate the non-F surface. The horizontal axis shows the various erosive acids, and the longitudinal axis shows Ld. From the surface prior to demineralization experiment, the depth of demineralization was determined up to a site showing 95% healthy dentin. For each sample, five sites were measured. All five samples were measured and the mean ± SD is shown. When all the four types of acid caused by erosion were compared between the experimental surface with fluoride treated and the non-F surface with was not fluoride treated, the Ld of the experimental surface was small.
EPMA analysis

The results of the qualitative EPMA from the sample surface to a given depth of fluorine, calcium, and phosphorus are shown in Fig. 8. Dotted line means a position of dentin surface. The line analysis revealed that the dentin surface layer at 20 µm from the measurement starting point showed fluorine, calcium, and phosphorus peaks (Fig. 8). Fluorine was distributed only in the 20-30 µm surface layer of dentin and could hardly be detected at depths of 30 µm onwards (Fig. 8A). The intensity peak in the surface layer was approximately 15-20 fold of the deep layer, which was markedly high. Calcium maintained an intensity of around 1,000 cps even in layers at depths of 20 µm onwards, and the largest peak was seen in the 20-30 µm surface layer of dentin (Fig. 8B). The site of the maximum calcium peak matched the peak and depth of the F element (Fig. 8A, B). Phosphorus showed a behavior similar to that of calcium, as the intensity at 20 µm onwards was stable at around 600 cps (Fig. 8C). The mapping image of the fluorine, calcium, and phosphorus elements in the EPMA surface analysis from the sample surface to a depth of 30 µm as with the scanning electron microscopy (SEM) image is shown in Fig. 9. Fluorine was located in a very thin range of 2 µm from the dentin surface layer and up to a depth of 15 µm from the surface layer of the dentinal tubule in the tubular wall (Fig. 9B). The intensity of fluorine in the dentinal tubular wall up to about 5 µm from the surface layer was comparatively strong, weakened with increasing depth from the surface, and could hardly be detected at depths of 15 µm onwards (Fig. 9B). The location of calcium and that of phosphorus matched and had a strong and constant intensity regardless of depth from the surface layer (Fig. 9C, D).

XPS analysis of the dentin surface after topical fluoride application

The results of the qualitative analysis of the XPS analysis that was performed to identify substances formed on dentin surface following the topical fluoride application are shown in Fig. 10. The analytical substances detected from the broad spectrum were carbon, calcium, phosphorus, fluorine, nitrogen, and oxygen. The binding energy of carbon (C1s) was confirmed to have a biphasic peak at 285 eV and 288 eV and separation of the peaks revealed the presence of N-C=O and (CH2)n, which are characteristic of collagen. The binding energy of calcium (2p3/2) was 348 eV and matched that of the CaF2 and CaCl2 peaks. Similarly, the binding energy of P was 134 eV, and both the HAp peak and F binding energy were 685 eV, which matched the CaF2 peak value. The nitrogen binding energy was 400 eV and matched the amino base (-NH2) of protein and collagen. The binding energy of oxygen was 532 eV and matched the peaks of CaCO3 and HAp.

Figure 8. Qualitative analysis of sample surfaces by electron probe microanalysis (EPMA). The horizontal axis shows the depth and the longitudinal axis shows the intensity (cps). From the top are the qualitative analysis results of fluorine, calcium, and phosphorus. The dotted line in the graph shows the position of the sample surface. The conditions of the qualitative analysis were acceleration voltage of 10 kV and irradiation current of 2 × 10^-8 A. The largest peak of calcium was seen in the 20-30 µm surface layer of dentin. The site of the maximum fluorine peak matched the peak and depth of the calcium element. Phosphorus showed a behavior similar to that of calcium, as the intensity at 20 µm onwards was stable at around 600 cps. The partial decrease in intensity peak indicated dentinal tubules.

Figure 9. Surface analysis of SEM image, Fluorine, Calcium, and Phosphorus by electron probe microanalysis (EPMA). SEM image (A), fluorine (B), calcium (C), and phosphorus (D) are shown. The scale bar represents 5 µm. Fluorine was located in a very thin range of 2 µm from the dentin surface layer and up to a depth of 15 µm from the surface layer of the dentinal tubule in the tubular wall. The location of calcium and that of phosphorus matched and had a strong and constant intensity regardless of depth from the surface layer. The black circular structure on the images were dentinal tubules.
**Discussion**

*Compounds produced on the sample surface*

After treatment with erosive acids, surface roughness of the experimental surface that had been pretreated which topical fluoride was increased significantly as compared to the untreated non-F group (Fig. 2). The same result was seen regardless of type of acid used but was most marked with acetic acid. The roughening of the surface after topical fluoride application demonstrated that a large amount of fluorinated compounds was formed on the surface of the experimental surface. It be-

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**Figure 10.** Surface analysis by X-ray photoelectron spectroscopy (XPS) analysis. The horizontal axis shows the binding energy (eV) and the longitudinal axis indicates the intensity (cps). The left column represents the spectrum of carbon, phosphorus, and nitrogen. The right column shows the spectrum of calcium, fluorine, and oxygen. Surface analysis was performed with Al-Kα as the X-ray source at 15 kV, 10 mA using an XPS analyzer. The spectrum of each element obtained was corrected by hydrocarbon-binding energy, where C1s = 285.0 eV. The analytical substances detected from the broad spectrum were carbon, calcium, phosphorus, fluorine, nitrogen, and oxygen. XPS qualitative analysis of dentin surface showed that the binding energy of calcium (2p3/2), 348eV, matched that of CaF2 and CaCl2. Moreover, 685eV, which is the binding energy of fluorine, matched the peak value of CaF2, thus demonstrating CaF2 formation.
came evident that, when APF acts on enamel, the free fluoride ion reacts with calcium supplied from the tooth to form CaF$_2$\(^{2-}\). Moreover, Koulouride et al. reported that Brushite, a crystal other than hydroxyapatite, and Whitlockite, a form of calcium phosphate, can be seen after the demineralization of enamel and are among the compounds formed during the remineralization process\(^{17}\). Moreover, in case of decalcification enamel, Chow and Brown suggested that CaF$_2$ and FAp could be formed if solutions containing appropriate amounts of F$^{-}$ and PO$_4^{3-}$ ions\(^{40}\). Although these previous studies used enamel as the sample, 98% of enamel and 70% of dentin is made up of hydroxyapatite, and the mechanism of action of applied APF is applied is not different\(^{49}\).

Therefore, when APF is applied to the dentin surface, elution of Ca$^{2+}$, HPO$_4^{2-}$, and OH$^{-}$ ions occur due to demineralization by the phosphoric acid present in the APF. The eluted Ca$^{2+}$ reacts with the F$^{-}$ supplied from APF to form calcium fluoride. This does not contradict the fact that in the EPMA qualitative analysis, the fluorine element was distributed only in 20-30 µm of the dentin surface layer, and the strong peak on the surface layer was approximately 15-20 fold of the deep layer, which is markedly high, and the position of the maximum peak of calcium matched the peak and depth of fluorine (Fig. 8A, B). Moreover, surface analysis also confirmed that the location of fluorine matched that of calcium (Fig. 9A, B). XPS qualitative analysis of dentin surface showed that the binding energy of calcium (2p3/2), 348 eV, matched that of CaF$_2$ and CaCl$_2$. Moreover, 685 eV, which is the binding energy of fluorine, matched the peak value of CaF$_2$, thus demonstrating CaF$_2$ formation (Fig. 10B, D). The amount of calcium fluoride on the tooth surface is reported to affect acid resistance\(^{20}\). By comparing the control surface and the demineralization-treated surface, we found that topical fluoride application decreased the substance defect caused by all but phosphoric acid (Figs. 3 and 4). We could confirm from observation of the CMR images that topical fluoride application suppressed demineralization with all four types of erosive acid. With phosphoric acid and citric acid, there was no distinct highly calcified layer; however, with lactic acid and acetic acid, a highly calcified layer was seen on the tooth surface (Fig. 5). In particular, with acetic acid, contrary to the demineralization observed below the surface, a broad band of hypercalcification came evident that, when APF acts on enamel, the free fluoride ion reacts with calcium supplied from the tooth to form CaF$_2$\(^{2-}\). Moreover, in case of decalcification enamel, Chow and Brown suggested that CaF$_2$ and FAp could be formed if solutions containing appropriate amounts of F$^{-}$ and PO$_4^{3-}$ ions\(^{40}\). Although these previous studies used enamel as the sample, 98% of enamel and 70% of dentin is made up of hydroxyapatite, and the mechanism of action of applied APF is applied is not different\(^{49}\).

The mechanism of reaction between fluoride and the dentin

Contrary to enamel, in dentin, dentinal tubules open to the surface and the compositional ratio of organic components is high. Thus, we performed a qualitative analysis using EPMA in order to clarify the behavior of fluoride after the topical fluoride application (Figs. 8 and 9). From the results of the line analysis, fluorine presented a large peak and two small peaks, at depths of 50 µm from the surface (Fig. 8). There was a marked decrease in calcium and phosphorus between the two peaks of fluorine, suggesting the presence of dentinal tubules. The fluorine peak was located only at the site of peritubular dentin, demonstrating uptake of fluoride ions into the peritubular dentin from the dentinal tubule wall. Surface analysis revealed that fluorine not only exists in the surface layer but is also abundantly present locally in the surroundings of dentinal tubules at a depth of approximately 20-30 µm below the surface (Fig. 9). In the report by Hirose et al., considerable deposition of fluorinated compounds was seen on the surface of dentin that had been treated with 2.0% NaF, and the deposition was mainly found between the dentinal tubules and the peritubular dentin. However, there was no blockade of the dentinal tubules\(^{21}\). It became evident from this study that the fluorine is distributed surrounding the dentinal tubule periphery and overlaps with the sites where calcium is present (Fig. 9). Thus, it was demonstrated that, when APF is applied to the tooth and thus to dentin, besides its action on the surface layer, it also induces the formation of calcium fluoride on peritubular dentin through the dentinal tubules, resulting in improved acid resistance.

From this study, it was evident that dentin to which APF had been applied showed increased resistance towards four types of erosive acids, namely, lactic acid, phosphoric acid, acetic acid, and citric acid. However, this study demonstrated that acid resistance is influenced by the type
of acid: while lactic acid and acetic acid display acid resistance by forming a calcified layer, citric acid, which has chelating effects, likely does not display adequate acid resistance. In addition, our findings implicate a mechanism by which calcium fluoride is formed on the tooth surface after topical fluoride application on dentin, and fluoride ions are taken up into the peritubular dentin through the dentinal tubule, resulting in improved acid resistance. Nevertheless, uniform application of APF on dentin for preventing tooth erosion is likely inadequate against acids with a chelating action. Thus, there is a need for developing a new tooth erosion prevention method that considers the effects of the various acid types.

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Conflict of Interest
The author have declared that no COI exisis.

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