

Root surface reactions to TiF_4 and SnF_2 solutions in vitro

An ultrastructural study

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Previous studies have indicated that TiF_4 and SnF_2 differ in their demineralizing effect when applied topically to root surfaces at the same acidity. The aim of this study was to examine in more detail the outermost layer of root surface specimens by transmission electron microscopy (TEM) after short-term exposure to these acidic fluoride solutions. Root surface specimens were exposed for 1 min and 4 min to equimolar (1.1 MF) solutions of a) TiF_4 , native pH 1.0; b) SnF_2 , acidified to pH 1.0; and c) SnF_2 , native pH 2.5. The specimens were then rinsed in saline and processed for TEM. Exposure to TiF_4 resulted in a partly demineralized zone 8–10 μm deep after 1 min and 5–27 μm deep after 4 min of application. A 0.1- μm -thick, electron-dense coating was present in all TiF_4 -treated specimens. Acidified SnF_2 resulted in a completely demineralized zone, 4–7 μm deep, when applied for 1 min and 4 min, whereas SnF_2 at native pH produced a 0.5- to 1.0- μm -wide partially demineralized zone. Unevenly distributed crystalline deposits were a frequent finding on SnF_2 -treated surfaces. The results indicate that solutions of TiF_4 and SnF_2 at native pH will cause only slight demineralization when applied topically to root surfaces. □ *Cariology; demineralization; fluorides, topical; transmission electron microscopy*

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Among different fluorides proposed as topical agents in caries prophylaxis are titanium tetrafluoride (TiF_4) and tin fluoride (SnF_2). The rationale for combining fluoride with Ti and Sn ions is, first, that the complex-binding ability of the metal ions enables them to bind concomitantly to fluoride and tooth tissue, thereby enhancing fluoride retention (1, 2). Second, acidic fluoride solutions may produce a slight surface demineralization and promote formation of HF and thereby facilitate penetration of fluoride into the tooth tissues (2, 3). Third, fluoride-metal complexes form surface coatings that are thought to give an additional caries-protective effect to treated tooth surfaces (4–10).

After application of aqueous TiF_4 and SnF_2 to root surfaces, considerable deposition of fluoride in the outer 50 μm has been

observed (7, 9–13). The demineralizing potential, however, seems to be different for the two agents. Even at the native pH 1 of the TiF_4 solution, demineralization of the root tissue has not been observed by the analytic method used, whereas the SnF_2 solution, when adjusted to the same pH, seems to produce considerable demineralization (7, 11).

In previous studies microradiography, polarized light microscopy, and electron microprobe analysis have been used. These methods do not enable a detailed examination of the outermost few micrometers of the root surface. Therefore, the aim of the present study was to examine in greater detail the very surface layer of root surface specimens by transmission electron microscopy (TEM) after exposure to TiF_4 and SnF_2 solutions.

Materials and methods

Five premolars extracted for orthodontic reasons were collected for the study and stored in 100% humidity in glass vials containing wet cotton and crystals of thymol. The teeth were scaled with curettes to remove soft tissue remnants, polished with pumice in a rubber cup, and rinsed in tap water. The crowns were discarded, and the roots divided in seven parts, each having original root surface on one side. One specimen from each root was immersed in equimolar solutions of either TiF_4 or SnF_2 at various pHs and for various periods of time (Table 1).

The solutions were prepared by dissolving the fluorides in distilled water to a molarity of 1.1 MF. Thus, the concentrations were 3.4% TiF_4 and 8.6% SnF_2 , giving native pHs of 1.0 and 2.5, respectively. For specimen groups E and F the SnF_2 solution was adjusted to pH 1.0 by adding HCl. After fluoride exposure the specimens were immersed three times in baths of 100 ml saline

for 2 min under continuous stirring and immediately processed for TEM. The pH of the saline was recorded before the washing procedure and after each bath.

The specimens were dehydrated in increasing concentrations of acetone, embedded in epoxy resin, and sectioned with diamond knives perpendicular to the root surface. Thin sections were collected on copper grids and examined unstained in a Jeol JEM-100 electron microscope. Micrographs were taken at magnifications of 6000 to 40,000 diameters from each specimen. The width of the demineralized surface zone was measured on photographic prints.

Results

The results are summarized in Table 2. Application of 3.4% TiF_4 (1.1 MF) produced a partly demineralized zone in the outermost 5–27 μm of the root surface in all specimens (Fig. 1). After 1 min application

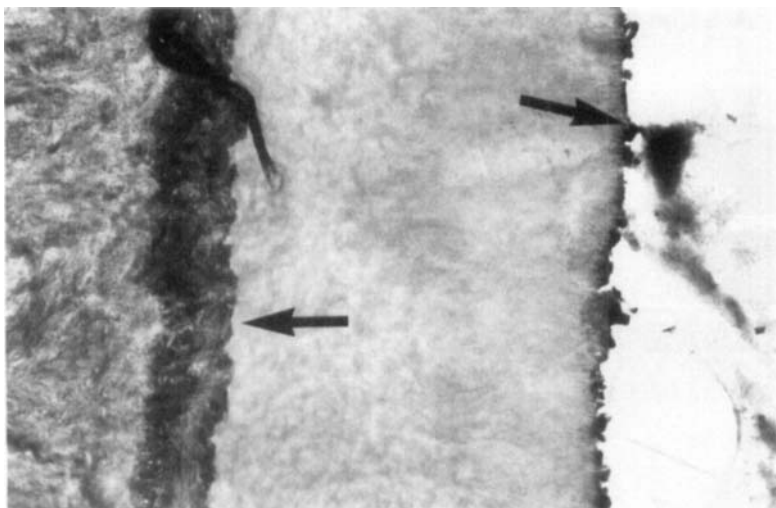
Table 1. Distribution of experimental material in accordance with fluoride agent, pH, and fluoride application period

Specimen group	Agent	pH	Application period	No. of specimens
A	TiF_4	1.0	4 min	5
B	TiF_4	1.0	1 min	5
C	SnF_2	2.5	4 min	5
D	SnF_2	2.5	1 min	5
E	SnF_2	1.0	4 min	3
F	SnF_2	1.0	1 min	3
G	Control		No treatment	3

Table 2. Number of specimens with surface changes, in relation to number of specimens treated and the character and depth of demineralization

Group	No. of specimens demineralized/treated	Character of demineralization	Depth (μm) of demineralized zone
A	5/5	Partial	5–27
B	5/5	Partial	8–10
C	1/5	Partial	3–5
D	0/5	—	—
E	3/3	Total	4–7
F	3/3	Total	4–7
G	0/3	—	—

Fig. 1. Root surface specimen treated with 3.4% TiF_4 for 1 min. A partly demineralized surface zone 6–7 μm wide, and an outer electron-dense coating are present (arrow). Subjacent to the demineralized zone is a hypermineralized zone (arrow). (Electron micrograph; magnification, $\times 6000$.)



the demineralization zone did not exceed 10 μm in depth, whereas after 4 min demineralization up to 27 μm in depth was recorded.

An approximately 0.1- μm -thick, electron-dense coating along the surface of the demineralized layer was a constant finding in the TiF_4 -treated specimens. Subjacent to the demineralized zone a highly electron-dense layer was often present (Fig. 1).

When applied for either 1 min or 4 min, 8.6% SnF_2 (1.1 M F) acidified to pH 1.0 resulted in almost complete demineralization

in a 4- to 7- μm -wide zone. An example is shown in Fig. 2. At the border zone between normal and affected dentin, partial removal of mineral crystals resulted in enhanced appearance of collagen cross-banding (Fig. 3). Closer to the surface very few mineral crystals could be distinguished.

SnF_2 solutions at native pH resulted in minimal change in the root surface when compared with controls (Figs. 4 and 5). In one specimen, however, a demineralized zone of 3–5 μm was observed (Fig. 6). At high magnifications partial dissolution of

Fig. 2. Root surface specimen treated with 8.6% SnF_2 acidified to pH 1.0 for 1 min. A nearly completely demineralized zone, 5–6 μm wide, is present. Fragmented electron-dense material is present at the surface of the demineralized zone (arrow). (Electron micrograph; magnification, $\times 6000$.)

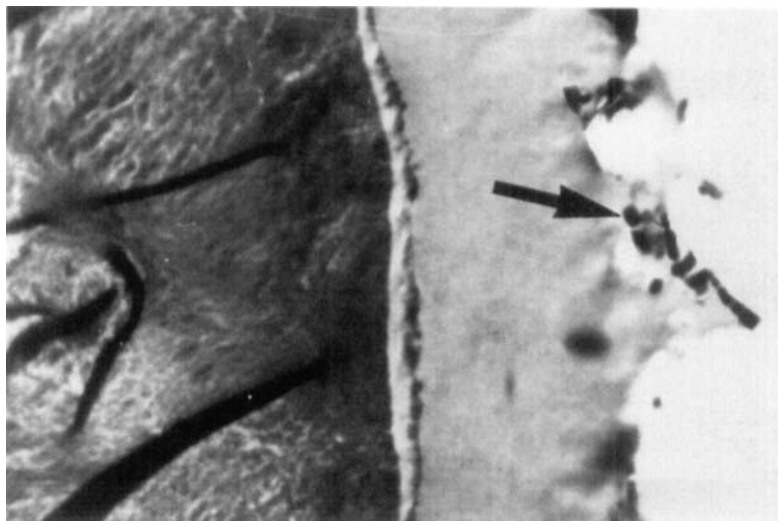




Fig. 3. Border between normal and demineralized dentin in a root surface specimen treated with 8.6% SnF_2 acidified to pH 1.0 for 1 min. Remaining mineral crystals in the demineralized zone enhance collagen periodicity (arrow). (Electron micrograph; magnification, $\times 40,000$.)

mineral crystals was observed in the outermost 0.5–1.0 μm in some areas (Fig. 7), but no distinct zone of demineralization could be observed.

Unevenly distributed aggregates of electron-dense material were often present on surfaces treated with native or with acidified SnF_2 solutions (Figs. 2, 4, and 6). The particles were somewhat fragmented by the sectioning procedure, and their true configuration could, therefore, not be ascertained.

The saline used for washing of the specimens in all groups showed a lowering in pH, with return towards base-line value after the third washing (Table 3).

Discussion

The results showed that topical application of TiF_4 or SnF_2 solutions at pH 1.0 had a demineralizing effect on root surfaces; the character of the demineralization, however,

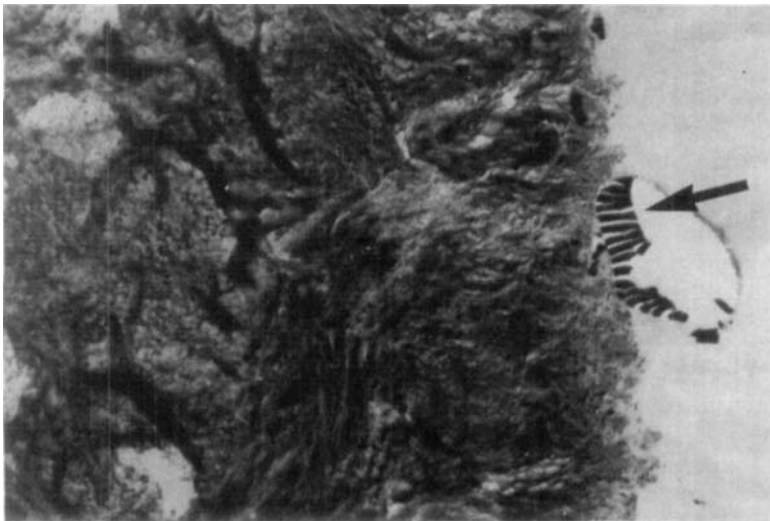


Fig. 4. Root surface specimen treated with 8.6% SnF_2 for 1 min. Electron-dense material is present on the surface (arrow). No distinct demineralized zone. (Electron micrograph; magnification, $\times 6000$.)

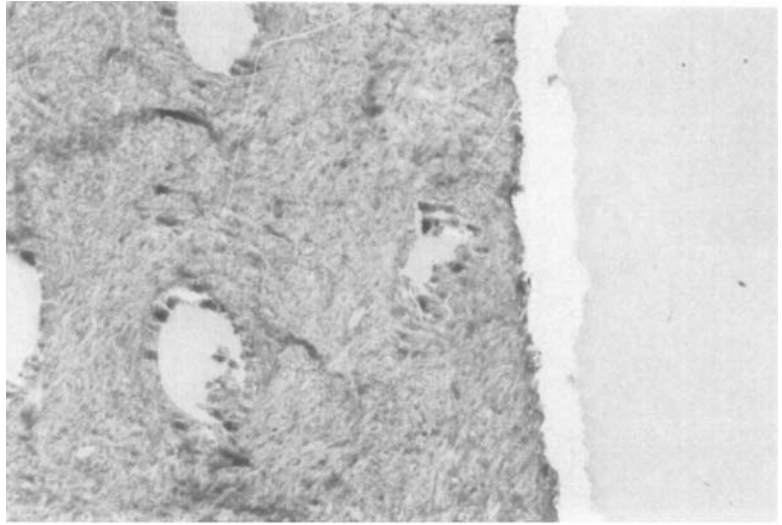


Fig. 5. Control specimen illustrating unaltered root dentin surface. (Electron micrograph; magnification, $\times 6000$.)

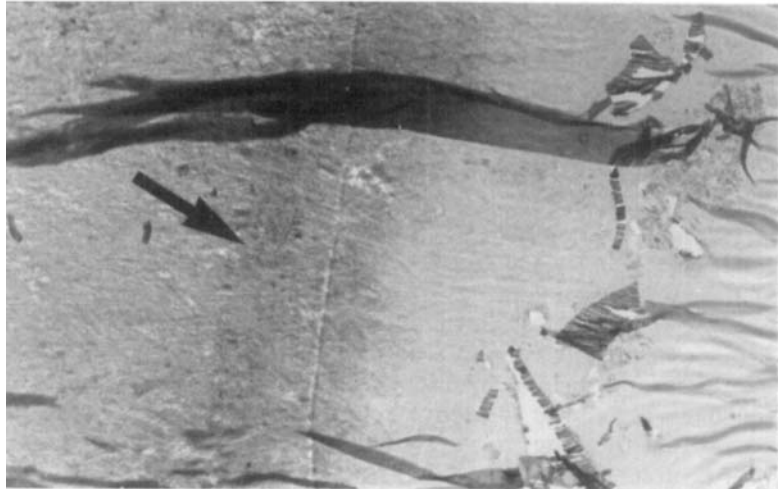


Fig. 6. Root surface specimen treated with 8.6% SnF_2 for 4 min. A partially demineralized surface zone 3–5 μm wide and subjacent hypermineralized zone (arrow) are present. (Electron micrograph; magnification, $\times 6000$.)

differed between the two agents. The complete demineralization resulting after application of acidified SnF_2 explains why this is more readily detectable by the electron microprobe technique as well as by micro-radiography (7, 11, 13).

The nearly complete lack of demineralization observed after application of the SnF_2 solution at native pH was more unexpected, since signs of demineralization of enamel and of dentin have previously been reported (13, 14), and root dentin is the more

Table 3. pH of washing solution after subsequent washings of three fluoride-treated specimens in 100 ml saline. Saline pH before bath was 5.4

Specimen group	Saline pH		
	1st bath	2nd bath	3rd bath
A	3.45	4.92	5.00
B	3.46	4.66	4.80
C	3.31	5.01	5.00
D	3.30	4.95	5.05
E	3.30	5.21	5.24
F	3.34	5.01	5.05

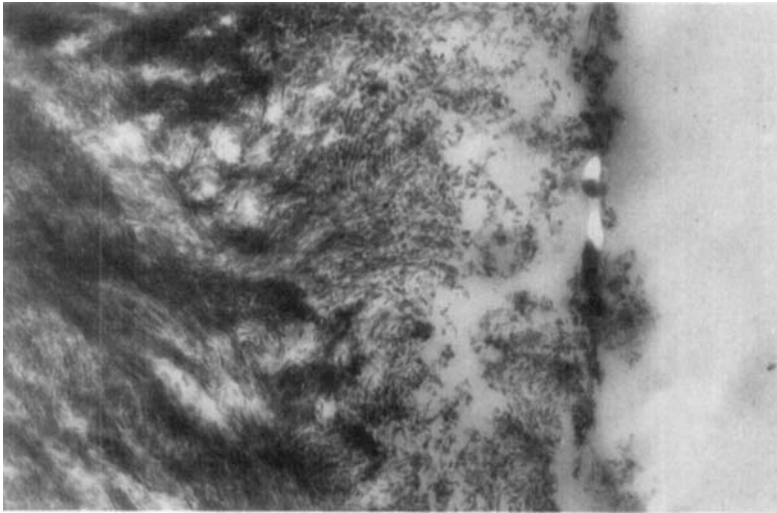


Fig. 7. Root surface specimen treated with 8.6% SnF_2 for 1 min illustrating partial dissolution of mineral crystals in the outer 0.5–1.0 μm . (Electron micrograph; magnification, $\times 45,000$.)

vulnerable of the two tissues (15). Theoretic considerations that may lend credence to the present findings have, however, been presented (7). Compared with the pronounced power of the Ti ion to expel a proton from water and thereby cause a low pH of the hydrolyzed TiF_4 solution, the Sn ion is assumed to have a limited affinity for the oxygen atom of water. This is reflected in the higher pH of the hydrolyzed SnF_2 solution than in the very acidic TiF_4 . When hydrolysis of metal fluorides results in a low pH, the metal is also expected to have a strong tendency to complex with phosphate on the tooth surface. Consequently, the titanium compounds formed are so tightly bound that they are not easily substituted with protons even at the high proton concentration present at pH 1.0. The binding of tin to phosphate is not as strong as that of titanium, but since the affinity for the oxygen atom of water is limited as well, the amount of dissociated protons will be too small to displace tin during a competition for the oxygen atom of the phosphate group. Tin ions will, consequently, adhere nearly permanently to mineral on the root surface at native pH. When the pH of the solution is reduced to 1.0, however, the more plentiful protons will displace tin, and the protonated phosphate groups on the root surface will go into solution and leave a demineralized surface.

Small surface craters characteristic of acid etch patterns have been reported after application of a 10% SnF_2 solution to enamel for 8 min (16). Apparently, the much shorter application periods used in the present study were not sufficient to produce a similar effect. The observed lowering of the pH in the saline used to wash the treated specimens, however, indicates that acid remained in the dentin and that both demineralization and remineralization processes may continue for a period of time after exposure to an acidic fluoride solution.

No attempt was made to identify the large crystalline structures observed on the surface of SnF_2 -treated specimens (Figs. 2 and 4). Most likely they represent a tin phosphate, possibly $\text{Sn}_3\text{F}_3\text{Po}_4$, since this substance has previously been proposed to occur together with calcium fluoride on enamel and dentin surfaces treated with SnF_2 (4, 14, 16–18). The uneven distribution of surface precipitates after SnF_2 application has also been noted previously (4, 16).

The acid-resistant Ti-containing coating consistently present on tooth surfaces after TiF_4 application (6, 19–21) may involve organo-metallic complexes, and its formation is probably favored by the rich organic matrix of the root tissues (19, 22). Hence, this coating possibly imparts an additional protective effect after topical application of TiF_4 to root surfaces.

Penetration of fluorides into dental hard tissues is enhanced if the surface is slightly demineralized before or in conjunction with the topical application (3). The minute surface changes observed after exposure to SnF_2 at native pH may therefore be considered advantageous. Acidified SnF_2 , by contrast, has a more marked demineralizing effect on the surface. Although a high fluoride uptake has been reported after topical application of acidified SnF_2 to roots in vitro (7), this agent may thus be less appropriate for clinical use. In the present study the acidified solution of SnF_2 was included to enable examination of the interaction of the two metal ions Sn and Ti with the root tissue at the same pH.

TiF_4 solutions, even at a pH as low as 1.0, produced only partial demineralization of a fine surface layer. This condition probably reflects the situation when Ti strongly competes for both the oxygen atom of water and the phosphate group of the mineral component. Apparently, the protons penetrate into the hard tissue only to a limited extent. This probably explains why the affected surface zone has not previously been detected by electron microprobe analysis or micro-radiography (7, 11, 13). The depth of the demineralized zones varied among specimens within each specimen group, presumably because of variations in the cementum thickness and in the width and number of dentinal tubules.

Even if the slight demineralization brought about by TiF_4 application under appropriate conditions may be expected to remineralize within a few weeks in the oral cavity (23–25), surface demineralization should in principle be kept at a minimum. The lowest concentration of acidic fluoride commensurate with a high reactivity with the root surface would therefore be preferable for clinical use. A 1.0% solution of TiF_4 produces a high and long-lasting fluoride concentration in root surfaces after an application period as short as 10 sec (10). Caries-inhibitory effects of TiF_4 at this concentration have been demonstrated both in rats and in a clinical study (26, 27). The rapid reaction with root tissues and the promising effect on enamel caries encourage further

investigation of TiF_4 as a topical caries-inhibitory agent in the clinical situation.

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