Effect of TiF_4 solutions on bacterial growth in vitro and on tooth surfaces

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The purpose of this study was to assess the antimicrobial effect of TiF_4 as compared with equimolar solutions of NaF, APF, and SnF_2 and to evaluate the effect, if any, on bacterial growth on topically treated tooth surfaces. In an in vitro study, paper discs impregnated with 20 µl of equimolar solutions of SnF_2, NaF, APF, and TiF_4 were placed on blood agar plates seeded with *Streptococcus mutans* and *Bacteroides gingivalis*. Sterile saline was used as control. Similar growth inhibition zones were found for all fluorides. In the second part of the study six volunteers carried intraoral appliances containing enamel and root surface specimens treated with 1% TiF_4 and untreated specimens for 18 h. Scanning electron microscopic examination of the experimental tooth surfaces showed great variation in bacterial growth between subjects, but no systematic difference between fluoride-treated and untreated specimens. Bacteria from test and control specimens were grown under aerobic and anaerobic conditions on blood agar and on mitis salivarius agar. Colony-forming unit counts showed great interindividual variations, but no differences could be observed between treated and untreated enamel or root surfaces. Thus, the hypothesis that the presence of a Ti-rich coating may influence bacterial colonization on TiF_4-treated tooth surfaces could not be substantiated.
□ *Caries prevention; dental plaque; fluorides; tetrafluoride; titanium*

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The mechanisms of the cariostatic action of fluorides are rather complex. Fluoride may render the tooth surface harder, more resistant to demineralization, and more prone to remineralization. In addition, a direct antimicrobial effect is supposed to play an important part for various fluoride salts, and polyvalent cations such as Cu^{2+}, Sn^{2+}, and Al^{3+} have been shown to have an antimicrobial effect both by themselves and when combined with fluoride (1–3).

When a clean tooth surface is exposed to saliva, a protein film, the acquired pellicle, is rapidly deposited before the formation of dental plaque (4). Differences in composition and rate of deposition between pellicles formed on native tooth surfaces and artificial surfaces indicate that selective absorption of salivary components to the tooth surface is a contributory mechanism in pellicle formation (5–7). Polyvalent cations may render the enamel more positively charged and hence increase the uptake of acidic pellicle material (8). The altered composition of the pellicle may subsequently influence the initial adsorption of bacteria and, thereby, the composition of early plaque on surfaces treated with polyvalent cations (9).

A caries-inhibiting effect of TiF_4 has been reported (10). Topical application of TiF_4 to dental enamel leaves a Ti-rich coating (11–14), which may change the reactivity of the tooth surface. Thus, TiF_4 treatment may conceivably affect the caries process not only by increasing the fluoride concentration at the tooth surface and by forming a fluoride reservoir but also by the possible effect of the deposited Ti-rich coating on the composition of early bacterial plaque both qualitatively and quantitatively.

A series of experiments was therefore performed to assess the antimicrobial effect of TiF_4 compared with more commonly used topical fluoride agents in vitro, and to evaluate the possible effect of this compound on bacterial deposition on dental enamel and root surfaces in vivo.

Materials and methods

Bacterial growth inhibition

Growth inhibition was tested in duplicate on human blood agar plates.

The bacteria used were *Streptococcus mutans*, strain 11367, and *Bacteroides gingivalis*, strain T64. Suspensions containing 10^4–10^5 bacteria/ml were flooded onto the surface of the plates, surplus amount was removed, and the plates were then dried before application of discs (15).

Paper discs (diameter, 4 mm) were impregnated with 20 μl of the following solutions: TiF_4 (1.1 and 0.32 M F), SnF_2, NaF, and APF (all 1.1 M F). Sterile saline served as control. The discs were dried before application to the plates.

The *S. mutans* plates were incubated at 37 °C for 24 h in air with 10% CO_2. The *B. gingivalis* plates were incubated in GasPak jars (GasPak Anaerobic System, Oxoid, London, U.K.) for 48 h. Growth inhibition zones around the discs were measured and their diameter recorded (in millimeters).

Bacterial colonization

Young caries-free premolars and third molars extracted for orthodontic reasons and stored in 100% humidity were used. The crowns were polished with pumice in a rubber cup to remove exogenous material, and the roots were curetted to remove soft tissue remnants. Circular enamel and root surface specimens, 4 mm in diameter, were prepared with a trephine bur and sterilized by 32-kGy gamma irradiation (Institute for High Energy, Kjeller, Norway).

Intraoral specimen holders were constructed for six test subjects. The specimen holder consisted of two lateral vestibular blocks of acrylic connected by a stainless steel labial bar and retained by clasps in the maxillary molar regions. Each acrylic block was fitted with four circular recesses in which standardized discs of either enamel or root dentin were attached with wax. For each subject two enamel and two root surface specimens treated with 1% TiF_4 for 1 min were fixed on the right side, one of each to be used for scanning electron microscopy and the other for bacterial cultivation. Untreated control specimens from the same teeth were fixed on the left side. The specimen holder was worn for an 18-h period, during which no oral hygiene procedures were allowed. The subjects had no restrictions in diet.

Table 1. Scoring of bacterial colonization on enamel or root surfaces by scanning electron microscopy

Observation	Score
Scattered single bacteria	1
Scattered microcolonies	2
Large aggregates of bacteria	3

Scanning electron microscopy. Test and control specimens of enamel and root surface with adhering deposits were removed from the specimen holder and immersed in 1% glutaraldehyde/4% formaldehyde in cacodylate buffer at 4 °C for 24 h. They were not washed but were moved briefly in the fixative to remove loosely adhering material. After postfixation in 1% osmium tetroxide in phosphate buffer for 2 h, the specimens were dehydrated in increasing concentrations of acetone, dried in air at room temperature, and then mounted on metal stubs with conductive silver paint. A 20-μm-thick coating of gold/palladium was evaporated onto the surface of the specimens under vacuum before they were examined in the scanning electron microscope (Philips SEM 515). Electron micrographs were taken at a magnification of ×700 in a central area of each specimen to assess the amount of deposit in accordance with the scoring system shown in Table 1. Electron micrographs at a magnification of ×3000 were taken in the same areas to verify the nature of the deposits.

Culturing. When removed from the mouth, the specimens for bacterial culturing were transferred directly to plastic tubes containing 0.4 ml sterile saline. The tubes were shaken for 30 sec in a vortex mixer (Whirli Mixer, Fisons Scientific Equipment, U.K.) to detach adherent bacteria. From each tube aliquots of 50 μl were plated out on human blood agar and mitis salivarius (MS) agar with a Spiral Plater, model D (Spiral System Inc., Cincinnati, Ohio, USA).

Table 2. Bacterial inhibition in accordance with fluoride or control solution and bacterial species. Diameter (in millimeters) of inhibition zone around impregnated paper discs. All figures represent means of duplicate experiments

Bacteria	TiF_4, 1.1 M F	TiF_4, 0.32 M F	SnF_2, 1.1 M F	NaF, 1.1 M F	APF, 1.1 M F	NaCl, 0.9%
S. mutans	28	14	28	32	29	0
B. gingivalis	72	55	74	77	74	0

One set of blood agar plates was incubated aerobically for 24 h, and one set and the MS agar plates were incubated anaerobically in GasPak jars for 48 h, all at 37°C. The blood agar plates were used to assess the total number of colony-forming units (CFU), whereas the MS agar is selective for oral streptococci.

Bacterial growth was routinely verified by gram-stained smears.

Statistical analysis

The compilation of results included tabulation of diameters of inhibition zones (Table 2), comparison of bacterial colonization (Fig. 1), and comparison of CFU counts on the different media (Fig. 4).

Because of the obvious lack of trends, further statistical analysis was not considered appropriate.

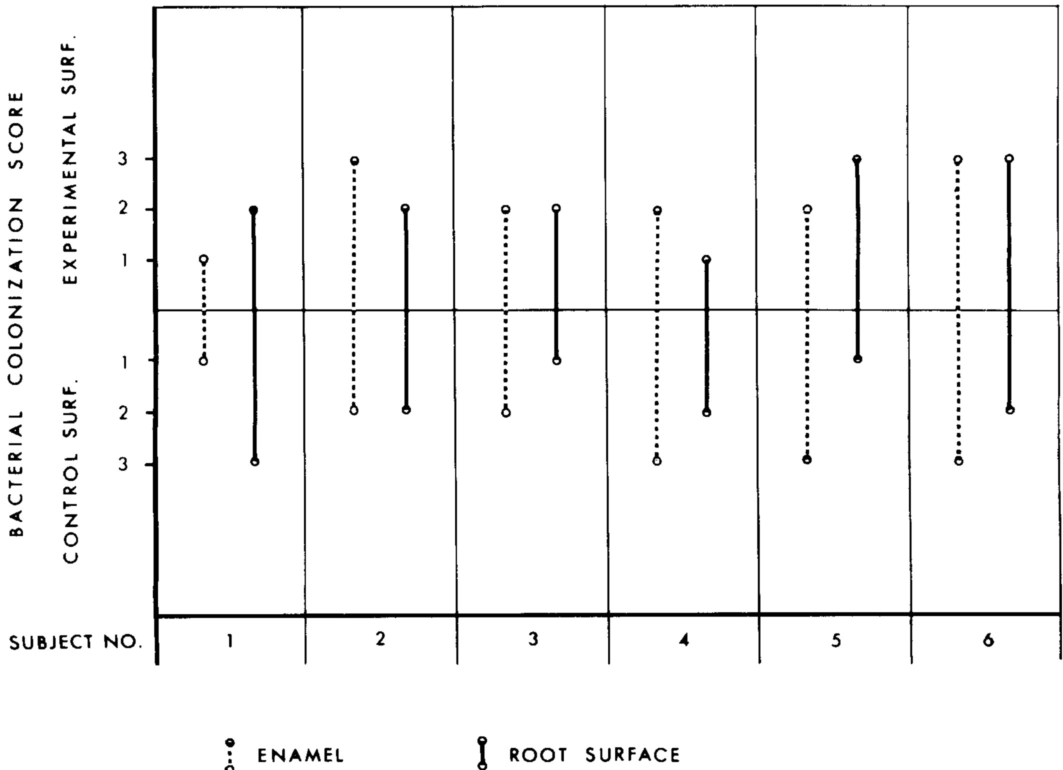

Fig. 1. Bacterial colonization on enamel and root surface specimens carried intraorally by six test subjects for 18 h in accordance with the scoring system presented in Table 1.

Results

Bacterial growth inhibition

The diameters of the bacterial growth inhibition zones around paper discs impregnated with fluoride or control solutions are listed in Table 2. The TiF_4 solution containing 1.1 M F seemed to possess antimicrobial properties to the same extent as SnF_2, NaF, and APF at the same molarity, and much more than the weaker (0.32 M F) TiF_4 solution.

Bacterial colonization

Scanning electron microscopy. The results from the scoring of bacterial deposits on TiF_4-treated and untreated tooth surfaces in vivo are presented in Fig. 1. The amount of deposit varied considerably both between individuals and between specimens. Systematic differences between TiF_4-treated and untreated specimens could, however, not be registered. Examples of different colonization scores are shown in Figs. 2 and 3.

Culturing. The colony-forming unit counts are illustrated in Fig. 4. No systematic differences in CFU counts could be registered between treated and untreated surfaces, neither for the enamel nor for the root specimens. Also, the pattern of colonization was similar irrespective of growth media.

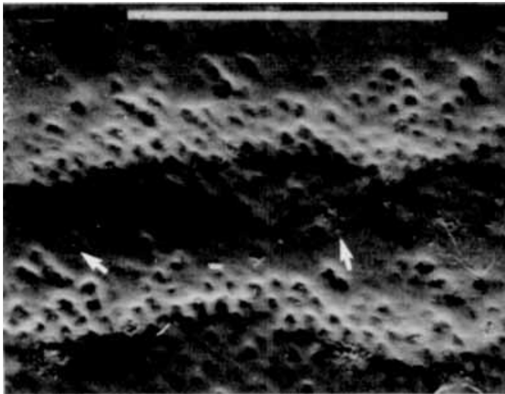

Fig. 2. Scanning electron micrograph illustrating score 1, scattered bacterial cells (arrows), on an untreated enamel surface after 18 h of oral exposure. Magnification scale indicates 0.1 mm.

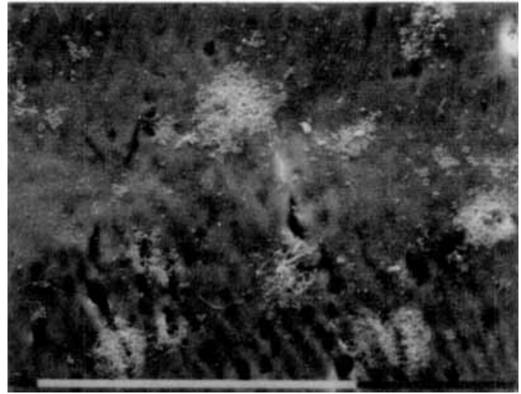

Fig. 3. Scanning electron micrograph illustrating score 3, large aggregates of bacteria, on an enamel surface treated with TiF_4 after 18 h of oral exposure. Magnification scale indicates 0.1 mm.

Discussion

This study showed that TiF_4, like several other fluoride agents, has an inhibiting effect on bacterial growth in vitro when applied in sufficient concentration and quantity. The minimal inhibitory concentration could, however, not be established by this simple experiment, nor could the relative effect of different pH values, different cations, and the fluoride itself.

In the clinical experiments plaque had formed both on TiF_4-treated and on untreated tooth surfaces. The large variations in the total amount of deposit could not be related to individual, to enamel versus root surface, to anaerobic versus aerobic bacteria, or to TiF_4-treated versus untreated surfaces. This means that the TiF_4 treatment did not significantly alter the properties of enamel and root surfaces with regard to plaque formation under the conditions of these experiments.

The experimental conditions differed from those in earlier studies, which have shown an inhibiting effect of fluorides on initial plaque formation. In those studies the agents were used at lower fluoride concentration and as mouth rinses rather than as topical agents (16–18). This reported effect on plaque formation could therefore include effects both on the overall oral condition and

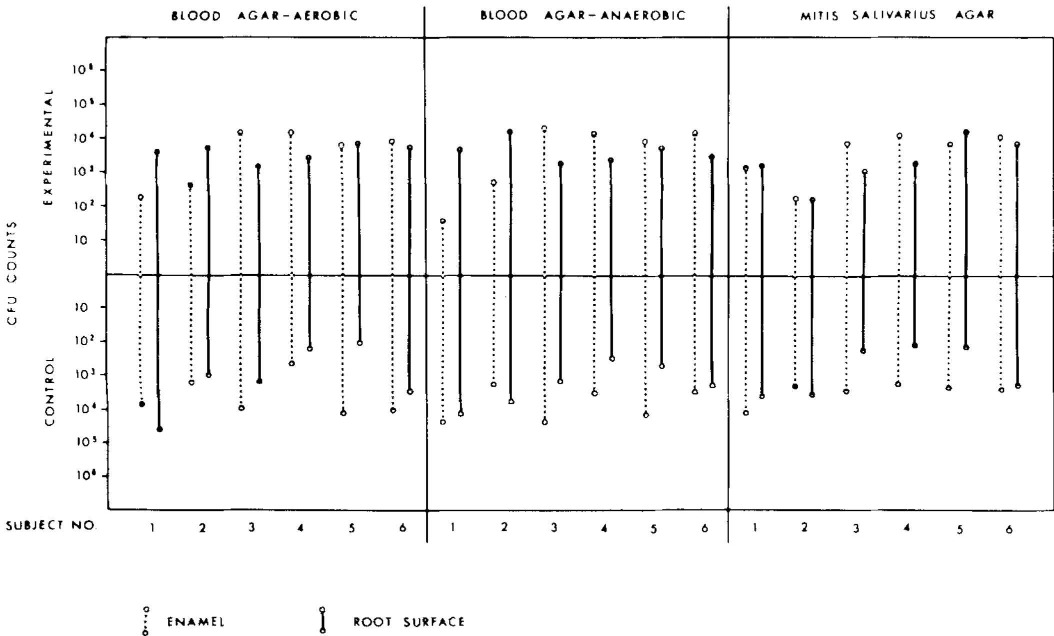

Fig. 4. Colony-forming unit counts (CFU counts) on enamel and root surface specimens for six test subjects in accordance with bacterial growth medium.

on the tooth surfaces. In the present study, however, the fluoride was applied, in higher concentration, to the tooth specimens before insertion in the mouth. Thus, as fluoride uptake in plaque from the oral environment was avoided, any observed effect could be ascribed to ions released from the test surface itself. Thus, the observed lack of effect on bacterial colonization indicates that the fluoride compounds formed a firm binding to the dental hard tissues with minimal amounts of fluoride released. The capacity of TiF_4 to bind to tooth surfaces has been discussed in greater detail in earlier studies (19–21).

The concentration of fluoride in the plaque in this study is not known. However, it is obvious that the minimal inhibitory concentration had not been reached.

In conclusion, the possible effect of a Ti-rich coating on bacterial colonization on enamel and root surfaces does not seem to be of clinical significance. Subtle differences cannot be ruled out and may be demonstrated by more meticulous methods, but neither scanning electron microscopy nor scoring of CFU counts could confirm the hypothesis that topical application of TiF_4 influences the formation of plaque on tooth surfaces.

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