

Stannous fluoride and its effects on oral microbial adhesive properties in vitro

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Abstract

The effect of sodium and stannous fluoride compounds on the coherence of Streptococcus mutans was examined. Both commercial and reagent grade preparations were tested on S. mutans, strain 6715. Test fluoride aliquots at various concentrations were added to sucrose suspensions of S. mutans at a starting microbial suspensions equivalent to 25% transmission as read in a spectrophotometer. Test samples were rotated at 35°, 80 rpm in an incubated orbital shaker. Analysis at 560 nm at various time periods, was performed to determine increases in %T as a function of aggregate formation. SnF₂ preparations routinely inhibited coherence at concentrations as low as .001%, whereas NaF showed no effects on microbial cell-to-cell coherence.

Prevention and control of dental plaque by daily rinsing with fluoride-containing solutions is widely used. The major efforts in fluoride mouth rinsing have been directed to treatment strategies that strengthen enamel or affect cariogenic bacteria. Although these fluoride effects traditionally have been considered to be mainly the result of a physiochemical interaction with enamel, there is evidence that fluoride also alters bacterial metabolism at low concentrations, and is bacteriocidal at higher concentrations (Loesche et al. 1975; Hamilton 1977). Therefore, the comparative qualities of various fluoride complexes on the oral microbial ecosystem has been a topic of interest over the years.

Konig (1959) studied the effects of topically administered stannous fluoride on the accumulation of plaque on rat molars in vivo. Not only was enamel solubility and caries reduced, but extensive plaque inhibition was noted as well. Tinanoff et al. (1976) further showed that there is more suppression of bacterial colonization on enamel by SnF₂ than by NaF with mouthrinses at a concentration of 100 ppm F, in vivo. Mouthrinsing was performed either once or twice a day for up to 7 days. The enamel specimens were

examined by scanning and transmission electron microscopy.

In a clinical study, White and Taylor (1979) also reported a significant reduction in plaque from daily rinsing with 0.1% SnF₂. Klock et al. (1985) further examined the effects of twice-daily mouthrinses with either SnF₂ or sodium fluoride (NaF) in adults and reported that after 1 year of rinsing with SnF₂ patients had fewer *Streptococcus mutans*/ml saliva, a lower incidence of caries, and less gingivitis than those participants rinsing with NaF. Long-term clinical studies by Leverett et al. (1986) suggested that 0.1% SnF₂ caused 4-5 times as much extrinsic stain as 0.05% NaF after a 28-month mouthrinsing period, but there were no statistically significant differences between the 2 fluoride groups in terms of total DMFS.

Early plaque formation is considerably enhanced by *S. mutans* species cohering to one another after adhering to a tooth surface. The cohesion-adhesion is at least partly due to glucan production and its coating of cells after sucrose utilization. These substances act as part of the biological "glue" enhancing bacterial adherence. Levan and other microbial components may also play a part in bacteria-to-bacteria binding.

The purpose of this study was to investigate stannous fluoride complexes on oral microbial adhesive properties and also to compare the stannous and the sodium ion forms of fluoride compounds.

Materials and Methods

Bacterial cultures of *S. mutans*, strain 6715, (American Type Culture Collection [ATCC] 25175, Lot 0585 S) were inoculated into a nutrient broth made of tryptose phosphate broth and Todd Hewitt broth 2:1 by volume and incubated 16-20 hr at 35° C. The bacterial cultures then were centrifuged at 3000 rpm x g for 10 min, after which the supernatant was decanted from the

solid pellet The pellet was mixed in a saline wash and centrifuged again for 10 min to remove residual spent media. All test and control solutions were maintained at pH 4.0 in phthalate buffer. This pH was used since SnF_2 was found not to autoprecipitate in the experimental time sequences required and microbial adherence was noted to be well promoted within the pH range 4.0-6.0 (Beierle et al. 1979). A volume of a 1.0% sucrose in 0.05 M phthalate buffer, pH 4.00 solution was added to the pellet which was resuspended to a concentration equivalent to 25% transmittance in a spectrophotometer (Model DB — Beckman; Irvine, CA) at 560 nm. Gel-Kam® (Scherer Lab Inc; Dallas, TX) was selected as representative of a stannous fluoride-containing product and ACT® (Johnson & Johnson Products Inc; New Brunswick, NJ) as a representative of a sodium fluoride-containing product. The SnF_2 rinse (Gel-Kam) was diluted with pH 4.00 phthalate buffer solution (1:3) immediately before use to produce a stock 0.1% solution. The NaF mouthrinse (ACT) was used as a stock solution at a concentration of 0.05%.

These starting stock solutions were used in testing as no interfering precipitation occurred in controls analyzed for the required 120 min necessary to complete the analyses. A 4.5-ml aliquot of the 25% T bacterial suspension made up in sucrose was added to 60 x 15-mm plastic Petri dishes (#1007 — Falcon Plastics; Oxnard, CA) and 0.5 ml aliquots of each fluoride-containing solution then were added to make a final volume of 5.0 ml. Varying dilutions of fluoride were made up to final concentrations from 0.01 to 0.005%. All test solutions were adjusted to a pH of 4.00 with phthalate buffer. Control 25% T suspensions of bacteria without fluoride additives were analyzed in 1% sucrose solutions or in with 0.85% saline solutions. The test Petri dishes were then rotated in a gyratory shaker (Model GS-8 — New Brunswick Environmental Shaker; New Brunswick, NJ) at 80 rpm at 35° C.

Triplicate samples of each group were analyzed in a spectrophotometer to assess bacterial aggregation at time points ranging from 0 to 120 min. All experiments were performed a minimum of 3 separate times in order to substantiate findings.

This aggregation assay is based on the detection of increased percentage of transmission as a function of increased microbial cohesion (Beierle et al. 1979).

The highest percentage of transmissions obtained was arbitrarily set to 100% and all other values then were corrected to 100% T as well, with a starting point of 0% T. Actually, all test samples started at 25% T which allowed for adequate microbial numbers for coherence to occur in the rotary suspension shaking procedure.

The mean value and standard deviation were calculated and plotted as corrected percentage of T vs.

time to determine characteristics of effects of fluoride-containing solutions on the aggregation of *S. mutans* (Fig 1).

Other aggregation assays were performed using chemically pure fluoride reagents in addition to the commercial product mixtures, ACT and Gel-Kam. The aggregation analyses usually were completed by 90 min at which time maximum cohesion was sustained.

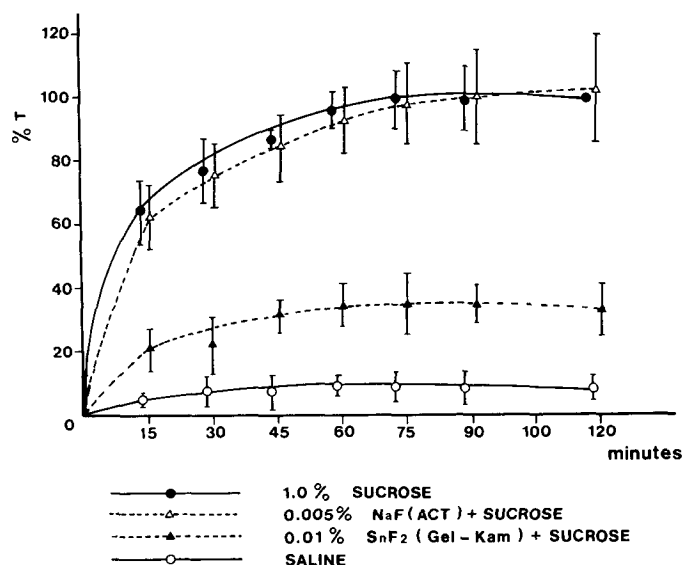


FIG 1. Aggregation profiles of *S. mutans* in the presence of sucrose, saline, and fluoride preparations.

1.0% sucrose
0.005% NaF (ACT) + sucrose
0.01% SnF_2 (Gel-Kam) + sucrose
Saline

Results

The coherence of *S. mutans* is greatly enhanced in the presence of sucrose compared to that of saline (Fig 1). Coherence was noted to start rapidly and the adherence assays generally were completed at the end of 1 hr. The addition of ACT solution containing 0.005% NaF showed no substantial differences over that of the sucrose control aggregation assay. The addition of Gel-Kam containing 0.01% SnF_2 , however, did reveal a significant reduction in the ability of *S. mutans* to cohere.

Photographs of the various aggregates detected after 60 min in Figure 1 show the various coherent masses (Figs 2a-d). The aggregates of *S. mutans* shaken in sucrose consistently were viewed as being compact, rounded, and distinctly visible, ranging from 1 to 2 mm in diameter (Fig 2a). When compared to samples rotated in saline (Fig 2b) the plates were seen to contain turbid, milky suspensions of nonaggregated bacteria. When these viable suspensions were viewed at 630x

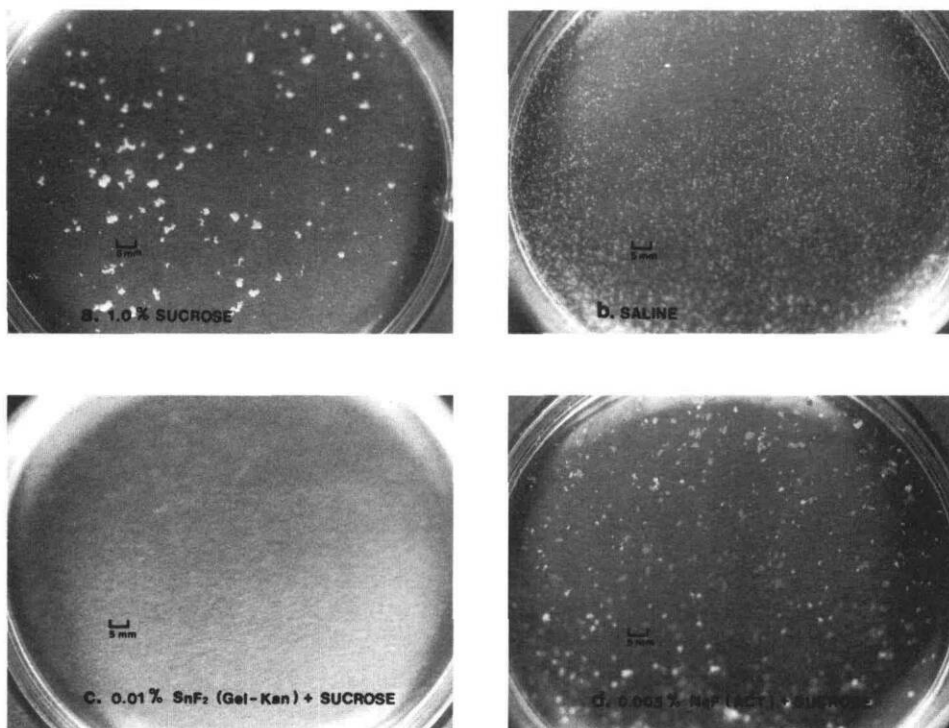


FIG 2. Aggregates of *S. mutans* at 60 min exposed to various reagents.

magnification by polarized light or interference microscopy the streptococci were found to be mainly in single cell suspension or in pairs. Bacteria rotated in saline barely cohered past that noted at the initial time of dispersion.

Starting cultures were dispersed by vigorous agitation on a vortex mixer followed by trituration through a narrow bore pipette. Bacteria shaken in the presence of SnF_2 revealed the presence of very small aggregates which never proceeded past the first few minutes of early coherence in the rotary shaker (Fig 2c). Those preparations tested in the presence of 0.005% NaF, however, showed that large aggregates of irregular form and dimension were formed (Fig 2d). Many of these aggregates were larger than those seen when *S. mutans* was tested in sucrose control solutions with no added fluorides. The aggregates formed in the presence of NaF also were less compact with extreme variability in size, ranging from relatively small to large.

In order to ascertain whether any effects were noted at different fluoride concentrations, studies were performed using varying concentrations of NaF, in the form of ACT (Fig 3). No difference in cohesion was detected over that of control cultures rotated in sucrose when *S. mutans* was exposed to NaF concentrations ranging from 5×10^{-3} to $1 \times 10^{-8}\%$. Reagent grade NaF also was made up in comparative concentrations and compared to the commercial mixture containing NaF (Fig 4, next page).

Again, no inhibition of cohesion of *S. mutans* was noted, even at concentrations higher than that found in stock solutions of ACT. Interestingly, *S. mutans* was continually noted to adhere slightly better in sucrose solutions in all concentrations tested ranging from 10^{-1} to $10^{-8}\%$ NaF.

Similar experiments were performed with Gel-Kam containing SnF_2 to determine if any differences existed when using the stannous vs. the sodium form of fluoride compound. The Gel-Kam preparation demonstrated reduction in coherence at SnF_2 concentrations as low as 0.001% (Fig 5, next page). This finding contrasted sharply with the lack of effect detected in preparations using NaF (Figs 3, 4). When reagent grade SnF_2 was utilized in place of the Gel-Kam preparation, similar effects on

cohesion in the same concentration range were noted (Fig 6, next page).

Discussion

The process of adherence of oral microbes to enamel surfaces or to various dental materials is of considerable interest (Fujioka et al. 1987). The coherence of homotypic or heterotypic microbial populations is also of importance in the accumulation of plaque flora and all its interactive accumulations and byproducts. The

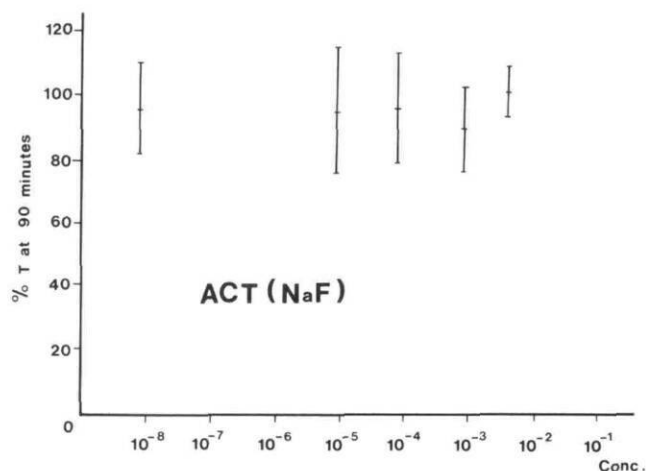


FIG 3. Aggregation of *S. mutans* at 90 min as a function of concentration of sodium fluoride in ACT mouthwash solutions.

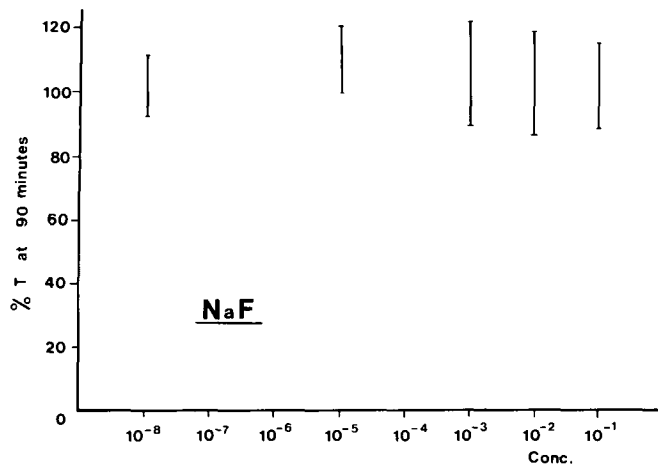


FIG 4. Aggregation of *S. mutans* at 90 min as a function of concentration of reagent grade sodium fluoride.

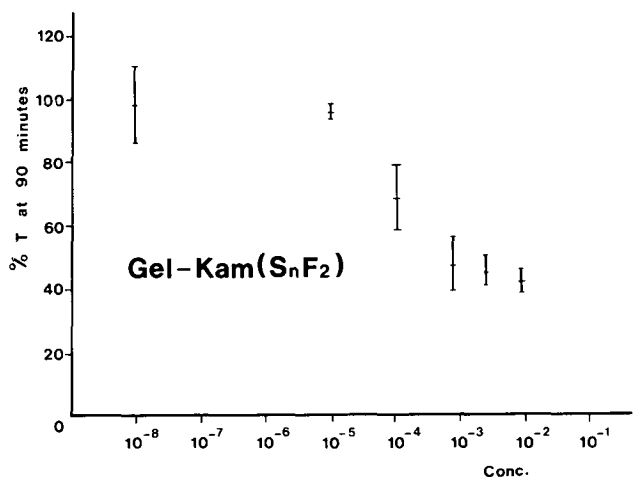


FIG 5. Aggregation of *S. mutans* as a function of stannous fluoride in Gel-Kam mouthwash solutions.

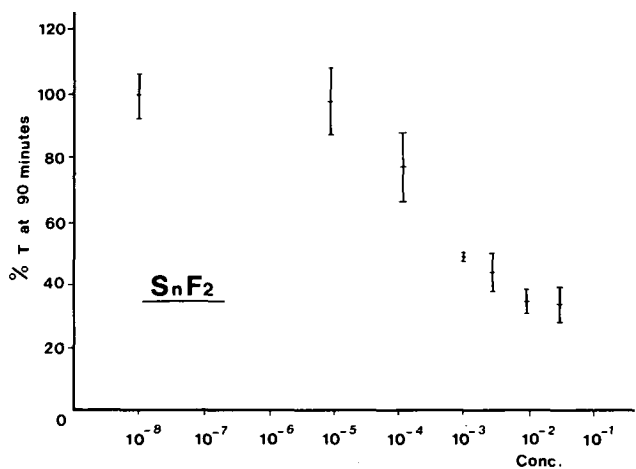


FIG 6. Aggregation of *S. mutans* as a function of concentration of reagent grade stannous fluoride.

effects of various fluoride compounds on either of the above processes, therefore, has added other dimensions to the understanding of the multifaceted roles of fluoride and the many available fluoride complexes. Zameck and Tinanoff (1987), have demonstrated that several strains of oral streptococci were inhibited in acid production by both NaF and SnF₂, whereas only SnF₂ was noted to decrease growth in all strains. A selective action was noted in the production of alkali-soluble glucan in *S. mutans*, while only SnF₂ enhanced alkali-soluble glucan production in the other oral streptococci examined. The effects of various fluoride compounds appear to vary based on the microbe examined, the conditions set for the experiment, and on the metabolic event examined.

The effect on cohesion of *S. mutans* was further noted whether or not one used commercial preparations containing a mixture of substances or purified preparations. The tin ion may add an additional feature to fluoride's effect on cohesion.

The question remains open as to whether the mechanisms underlying cohesion significantly differ from those of adhesion. The glucan coating may not be the only adherent molecules involved in the cohesion process and what other effects the tin ion has on metabolic requirements for cell-to-cell adhesion is not understood. Furthermore, these studies were run in the absence of saliva and its associated variables and this, as in any in vitro test system, cannot fully mimic the actual in vivo situation. Using probes of different metal ions in conjunction with fluoride can enhance our overview of the process of plaque production and microbial adherence.

The role of the tin ion in altering microbial metabolic functions and affecting bacterial growth, attachment, or acid production long has been an issue of interest and discussion (Wright and Jenkins 1954; Tinanoff et al. 1976; Treasure 1981). There is little doubt that the cumulative effects of fluoride complexes, at proper dose levels or pH range, serve as an excellent control vehicle over oral bacteria. A recent study examined the reduction of early plaque formation and gingival bleeding after exposure to a variety of agents, including acidulated stannous fluoride and zinc acetate. Both agents significantly reduced plaque accumulation and gingivitis (Hefti and Huber 1987).

Our in vitro findings indicate NaF has some as yet, nebulous effect on cohesion of *S. mutans* as viewed by light microscopy. We have visually noted that microbes cohered into large amorphous masses, but without the compactness and order of those aggregates rotated only in the presence of sucrose. On viewing cohesion of *S. mutans* in the presence of SnF₂, however, one noted only the presence of very small aggregates as well as greatly

reduced aggregation curves, compared to sucrose controls. As noted with our stannous fluoride studies as well as all of the other studies cited, the tin ion, when complexed with fluoride, adds additional impact to the fluoride compound. The synergistic or added inhibitory effect of the tin ion when complexed to fluoride may be a reality, but the actual function and mechanism of action requires further study. The observed effects on cohesion noted with SnF₂ in this study is consistent with other in vitro findings reported (Yost and Vandemark 1978; Berson et al. 1979). These in vitro observations also are consistent with numerous other in vivo studies reported (Muhler and Day 1950; Mercer and Muhler 1972; Stookey and McDonald 1974).

These experiments were run at pH 4.0 as the solubility of SnF₂ was found to be maximized at this pH (Beierle et al. 1979) while precipitating at higher pH levels. *S. mutans*, however, has limited growth capabilities at such low pH ranges (Bowden and Hamilton 1987).

Clinically, the anti-binding properties of stannous fluoride may aid patients wearing fixed orthodontic appliances, as elevated levels of *S. mutans* often have been found in these individuals (Corvett et al. 1981; Mattingly et al. 1983; Scheie et al. 1984). Patients undergoing oral surgery also may be considered candidates for SnF₂-containing mouthrinses where rigorous tooth brushing is not recommended, and patient care and oral health must be maintained. Further studies expanding in vitro findings into clinical situations would enlighten these findings.

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