An Experimental Gingivitis Study to Evaluate the Clinical Effects of a Stannous Fluoride Dentifrice

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Purpose: A double blind, controlled, parallel group trial utilizing the experimental gingivitis model was performed on thirty young adults to evaluate the clinical effects of a 0.45% stannous fluoride dentifrice used as a slurry on dental biofilm formation and the development of gingivitis.

Material and Methods: Following a thorough examination and oral prophylaxis procedures, subjects were randomly assigned to apply one of the following dentifrices twice daily over a three-week period: A) dentifrice slurry without active ingredients; B) 0.45% stannous fluoride gel; and C) *Colgate® Total* dentifrice slurry (0.30% triclosan, 0.24% sodium fluoride, 2% copolymer).

Results: After three weeks, the stannous fluoride dentifrice significantly ($p < 0.05$) reduced gingivitis compared with the *Colgate® Total* group by 39.7%. Gingival bleeding was also reduced relative to the *Colgate® Total* group. This difference was statistically significant ($P < 0.05$). During the experimental period, the mean PII scores increased almost linearly in all three groups without yielding any statistically significant differences.

Conclusions: The results of this clinical trial demonstrated that, over a three-week period, the application of a 0.45% SnF2 gel significantly inhibited the onset of gingivitis compared to Triclosan/sodium fluoride/copolymer (*Colgate® Total*). However, neither stannous fluoride nor Triclosan/sodium fluoride/copolymer (*Colgate® Total*) possessed sufficient antimicrobial activity to suppress biofilm formation in the absence of regular oral hygiene practices.

Key words: prevention, gingivitis, stannous fluoride, dentifrice, plaque, discolorations

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The primary role of biofilm formation is well established as an etiologic factor for both caries and periodontal diseases, and the importance of effective plaque control has been stressed for many decades (Löe et al, 1965). However, the majority of the population appear to have difficulty maintaining adequate standards of oral hygiene over prolonged periods of time. Hence, efforts have been made to supplement regular tooth cleaning with the application of chemical agents such as mouthrinses and dentifrices to improve oral health status (Lang and Brecx, 1995).

Due to the addition of fluorides into drinking water and table salt, as well as the widespread use of fluoridated toothpastes, a dramatic decrease in dental caries has been noted during the past three decades (Brunelle et al, 1982, 1990). Furthermore, epidemiological and demographic studies have shown a change in the pattern of oral diseases within Western industrialized populations (Lang et al, 1990; Brown et al, 1990). However, despite

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generally improved oral health, a high prevalence of gingivitis persists. This suggests that oral hygiene regimes remain inadequate.

In an effort to improve both preventive and therapeutic outcomes, extensive investigations led to the development of antimicrobial agents, which were recommended as adjuncts to mechanical plaque control (Kornman, 1986). In that respect, a cationic bisguanide, chlorhexidine digluconate was introduced (Löe and Schiøtt, 1970), and, owing to its undisputed clinical efficacy, this compound became the gold standard for the entire concept of chemical plaque control (Lang and Brecx, 1986). By virtue of its cationic nature, chlorhexidine is not easily incorporated into dentifrices owing to its interaction with other ingredients such as anionic detergents (Gjermo and Rølla, 1971). Side effects include: a bitter taste; the development of an extrinsic stain on teeth and tongue (Löe and Schiøtt 1970; Flötra et al,1971); alterations in taste perception of salt (Lang et al, 1988) and occasionally, increased calculus formation (Löe et al, 1976); and the development of desquamations of the gingivae and mucosae (Flötra et al, 1972). In order to reduce the side effects, particularly the development of stain, several other oral antiseptics have been investigated (Siegrist et al, 1986; Hefti and Huber, 1987). Also, various therapeutic dosages and concentrations were studied to minimize the side effects (Grossmann et al, 1986; Segreto et al, 1986).

Fluorides have also been advocated, not only for their effects on dental caries, but also as antiseptics in the context of chemical plaque control (Loesche, 1976). Amongst others, stannous fluoride (SnF_2) was promoted as a potential adjunct in the prevention of periodontal disease (Mazza et al, 1981). When incorporated into a dentifrice, stannous fluoride showed some antimicrobial and hence, antiplaque properties (Svatun, 1978). Nonetheless, the clinical effects of the stannous fluorides were clearly below those documented for chlorhexidine (Svatun et al, 1977; Hefti and Huber, 1987).

Stannous fluorides have been applied as mouthrinses (Tinanoff et al,1980), controlled release devices (Tinanoff et al, 1986), gels (Tinanoff et al, 1989) and dentifrices (Beiswanger et al, 1995, 1997; McClanahan et al, 1997; Perlich et al, 1997). Stannous fluoride dentifrices have a well-studied anticaries effect (Tinanoff, 1990) and seem to offer desirable antimicrobial properties (Tinanoff 1990, 1995), with some promising results in several studies of efficacy reducing biofim formation as well as gingivitis. In comparative studies (Binney et al, 1997; Riep et al, 1999), the intrinsic antimicrobial effect of SnF2 appeared stronger than observed for numerous other agents, i.e. essential oils and triclosan. However, until recently, lack of stability was a key obstacle for product marketing. Aqueous environments favored hydrolysis and oxidation of the stannous ion. Nevertheless, new formulations with stabilized stannous fluoride were developed recently, bringing the agent back in focus for use as an effective antimicrobial adjunct in prevention and possibly therapy.

The purpose of the present study was to evaluate the clinical effects of a 0.45% stannous fluoride dentifrice used as a slurry on dental biofilm formation and the development of gingivitis utilizing the experimental gingivitis model (Löe et al, 1965).

MATERIAL AND METHODS

Patient Selection

The study was approved by the Ethics Committee of the Canton of Berne, Switzerland. Thirty dental students or dental assistants (aged 21–28 years) from the University of Berne School of Dental Medicine were recruited for this three-week clinical trial. All volunteers were informed about the purpose, risks and benefits associated with the study and signed consent forms.

To be accepted for the trial, subjects had to have at least 24 teeth. They agreed to refrain from using any non-study dentifrice or mouthrinse and to limit use of chewing gum to 2 sticks per day for the duration of the trial. To qualify, the students were required to be generally healthy (no antibiotics within seven days of the recruitment day). Students were withdrawn from the study if they showed evidence of oral pathoses that required immediate treatment or obvious signs of periodontal disease.

Study Design

The study was designed as a double blind, controlled, parallel group trial utilizing the experimental gingivitis model (Löe et al, 1965).

Pre-experimental period: 21 days prior the start of the study (recruitment), initial levels of supragin-

gival plaque, gingivitis, gingival bleeding and oral soft tissue status were determined. After these examinations, all participants received thorough scaling and prophylaxis procedures performed by registered dental hygienists, and were instructed in optimal oral hygiene procedures including the Bass (1955) tooth brushing technique and interproximal cleaning. For three weeks, the subjects had to perform optimal oral hygiene to attain Plaque and Gingival Indices approaching zero. Participants who did not attain these goals were excluded from the study prior to commencement of the experimental period. All subjects were encouraged to clean with Crest® cavity protection dentifrice (0.24% sodium fluoride) using electric or hand-operated toothbrushes.

Experimental period: The volunteers were divided into three groups of 10 participants. The method of placement into a group was by randomization. Three slurries, two with different fluoride compositions and one without an active ingredient, were distributed to the three groups (A, B, C). Neither the subjects nor the examiners knew which treatment a student was allocated at any time during the study due to uniquely labeled identical white tubes containing the test dentifrices. For a period of 21 days, subjects had to refrain from any oral hygiene. The students had to rinse under supervision 5 days a week with the freshly prepared slurries twice daily for 1 minute. At weekends, the products were provided for home use, and the participants had to rinse in the morning before 10 am and after 5 pm. Compliance was evaluated by assessing the volume of the rinsing solutions returned to the main station on the following Monday.

The study was conducted over a three-week period with examinations at baseline, Day 7, Day 14 and Day 21 of no-oral-hygiene. After the 3-week experimental period, all participants resumed optimal oral hygiene procedures. Then they were re-examined at the final visit (Day 35).

Test Products

The following mouthrinses were distributed to the three groups:

Group A:	Dentifrice without active ingredient
Group B:	0.45% stannous fluoride gel
Group C:	Colgate® Total dentifrice (0.30% triclosan, 0.24% sodium fluoride, 2% copolymer)

The slurries were prepared freshly each morning and afternoon. Slurry rinses consisted of 5 g experimental or market product and 15 g of water. The mouthrinses were given in small plastic measuring cups of 30 ml and mixed immediately prior to use. Subjects were instructed to rinse under supervision twice daily for one minute. At the weekends, students received separate containers for water and dentifrice for preparation at home.

Clinical Parameters

All oral examinations were conducted under dental clinic conditions employing good illumination, compressed air, mouth mirrors and periodontal probes. Measurements were carried out by experienced and calibrated clinical examiners, with one examiner always assessing the same parameter. Calibration tests yielded a reproducibility of 85% and 92% for single PII and GI scores, respectively.

At the start of every examination day, oral photographs were taken to document different stages of the oral health status. The following parameters were thus evaluated:

Oral Soft Tissue Health (OST)

Visual examinations of the teeth as well as surrounding structures like the oral mucosa, tongue, lips and perioral area were conducted on recruitment and at the final visit (Day 35) to determine the oral soft tissue health and to ensure that no damage attributed to product use had occurred. Observations of ulcerations, indurations or changes in the surface texture were recorded to be normal, within normal limits or abnormal.

Plaque Deposits

After drying the teeth with a stream of air, undisclosed plaque was assessed (BEP) according to the criteria of the Plaque Index System (PII) (Silness and Löe, 1964) on the mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual surface of each tooth (except third molars).

Gingival Status

Gingival health or gingivitis was assessed by one examiner (NPL) using the criteria of the Gingival Index (GI) (Löe and Silness, 1963) on all surfaces of all teeth. Presence or absence of Gingival Bleeding as described by the Löe and Silness (1963) GI = 2.3 was analyzed separately.

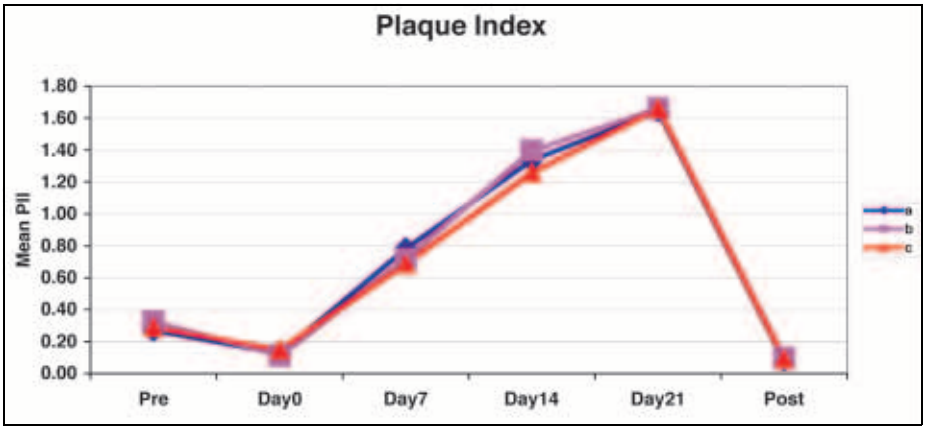

Fig 1 Mean Plaque Index for all treatment groups rinsing with either control (a), stannous fluoride (b), or Triclosan/sodium fluoride (Colgate® Total) (c) at all time intervals. There was no statistically significant difference ($p < 0.05$).

Statistical Analysis

Individual and group means and standard deviations as well as mean group frequencies of single index scores were calculated for all clinical parameters. Inter group comparisons at any given observation period were made using Student t-tests for independent samples and comparisons within the groups on a longitudinal basis were made using t-tests for dependent samples. The level of significance was set at $\alpha = 0.05$.

RESULTS

In each of the three test groups, 5 male and 5 female volunteer students in the age range of 21–28 years were recruited. All the 30 volunteers completed the experimental period. One volunteer was originally recruited, but had to withdraw from the study because of medical indications for antibiotic drug use immediately prior to the start of the study period.

At the pre-experimental examination, the three study groups demonstrated mean PII scores of 0.27 (SD ± 0.08), 0.33 (SD ± 0.13) and 0.29 (SD ± 0.08), respectively, with no statistically significant differences between any groups. The mean GI at this time was 0.20 (SD ± 0.16), 0.21 (SD ± 0.18) and 0.15 (SD ± 0.10), respectively. Again, there were no statistically significant differences between any group means. In assessing gingivitis by utilizing the proportions of GI = 2, the mean scores at the pre-experimental examination were 0.02 (SD ± 0.03), 0.02 (SD ± 0.02) and 0.01 (SD ± 0.02), respectively.

Oral Soft Tissue

Examination of the oral soft tissue health revealed no unexpected or clinically relevant adverse reactions associated with the use of the experimental rinsing solutions at any observation period.

Plaque Indices (Silness and Löe, 1964) (Fig 1)

At the beginning of the clinical trial (Day 0), all participants revealed mean plaque indices (PII) of: PII = 0.12 (SD ± 0.07) for Group A; PII = 0.11 (SD ± 0.08) for Group B; and PII = 0.15 (SD ± 0.09) for Group C. During the experimental period, the mean PI scores increased almost linearly in all three groups between Day 0 and Day 21. After the 3 weeks of no-oral-hygiene, the three groups showed a mean PII = 1.65 (SD ± 0.09) for Group A and a mean PII = 1.66 (SD ± 0.33, SD ± 0.09) for Group B and C, respectively (Fig 1). After resuming oral hygiene practices, the three groups yielded mean PII of 0.09 (SD ± 0.03), 0.10 (SD ± 0.04) and 0.09 (SD ± 0.03) for Groups A, B and C, respectively.

Gingivitis Scores (Löe and Silness, 1963) (Fig 2)

At the beginning of the trial (Day 0), all participants revealed mean Gingival indices (GI) of: GI = 0.06 (SD ± 0.03) for Group A; GI = 0.06 (SD ± 0.03) for Group B; and GI = 0.03 (SD ± 0.02) for Group C. During the experimental period, the mean GI scores increased in all three groups between Day 7 and Day 21. On Day 14, a mean GI = 0.32 (SD ± 016)

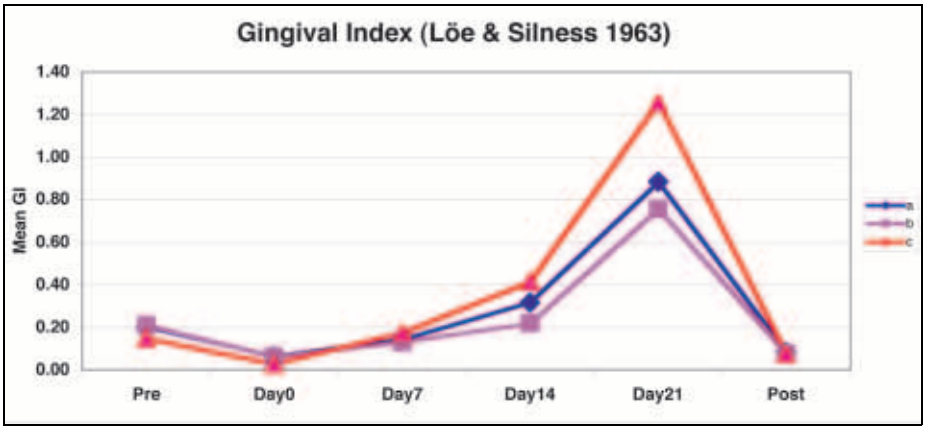

Fig 2 Gingivitis occurrence for all treatment groups rinsing with either control (a), stannous fluoride (b), or Triclosan/sodium fluoride (Colgate® Total) (c) at all time intervals. The stannous fluoride group is significantly lower ($p < 0.05$) than the Triclosan/sodium fluoride (Colgate® Total) (c) group.

was observed in Group A; a mean GI = 0.22 (SD ± 0.14) in Group B; and a mean GI = 0.42 (SD ± 0.27) in Group C. At Day 14, the difference of the mean GI between Group B and Group C was statistically significant ($p < 0.05$). After the 3 weeks of no-oral-hygiene, the three groups showed a mean GI = 0.88 (SD ± 0.23) for Group A; a mean GI = 0.76 (SD ± 0.24) for Group B; and a mean GI = 1.26 (SD ± 021) for Group C (Fig 2). At Day 21, the differences between the mean GI for Group B and Group C as well as between the mean GI for Group A and Group C were statistically significant ($p < 0.05$). After resuming oral hygiene practices the three groups yielded mean GI of 0.08 (SD ± 0.04), 0.07 (SD ± 0.03) and 0.07 (SD ± 0.03) for Groups A, B and C, respectively.

Gingivitis Scores assessed by GI = 2.3 (Fig 3)

At the beginning of the trial (Day 0), all participants revealed mean GI = 2 of 0.01(SD ± 0.01) for Group A; 0.01 (SD ± 0.01) for Group B; and 0.00 (SD ± 0.01) for Group C. During the experimental period, the mean proportion of GI = 2 scores increased in all three groups between Day 14 and Day 21. On Day 14, a mean of 0.03 (SD ± 0.03) was observed in Group A; a mean of 0.02 (SD ± 0.02) in Group B; and a mean of 0.05 (SD ± 0.06) in Group C. At Day 14, the there were no statistically significant differences between the mean proportions of GI = 2 scores of any groups. After the 3 weeks of no-oral-hygiene, the three groups showed a mean proportion of 0.23 (SD ± 0.11) for Group A; a mean proportion of 0.15 (SD ± 0.10) for Group B; and a mean proportion of 0.43 (SD ± 0.15) for Group C (Fig 3). At Day 21, the differences between the mean GI = 2 proportions for Group B and Group C as well as between the mean GI = 2 proportions for Group A and Group C were statistically significant ($p < 0.05$). After resuming oral hygiene practices the three groups yielded mean proportion of GI = 2 of 0.00 (SD ± 0.00) for all three groups.

DISCUSSION

The objective of this study was to evaluate the effects of a 0.45% stannous fluoride dentifrice on supragingival plaque and gingivitis development in comparison to a marketed Triclosan/sodium fluoride/copolymer containing toothpaste (Colgate® Total) and a control in an adult population.

The experimental gingivitis model (Löe et al, 1965) was chosen as a screening model to evaluate the efficacy of the experimental compound in delaying plaque formation and the development of gingivitis. In the control group (A) the mean Plaque indices rose to PII = 1.65 during a three-week period of undisturbed plaque accumulation. Consequently, generalized gingivitis with a mean GI = 0.88 developed. These values are in close agreement with results from previous studies that utilized the experimental gingivitis model (for review see Lang et al, 2002). In order to confirm the results obtained with the Gingival Index system (Löe et al, 1963), a second parameter was chosen to evaluate the gingival changes encountered during the experimental period. This modification of the GI was limited to the evidence of bleeding on

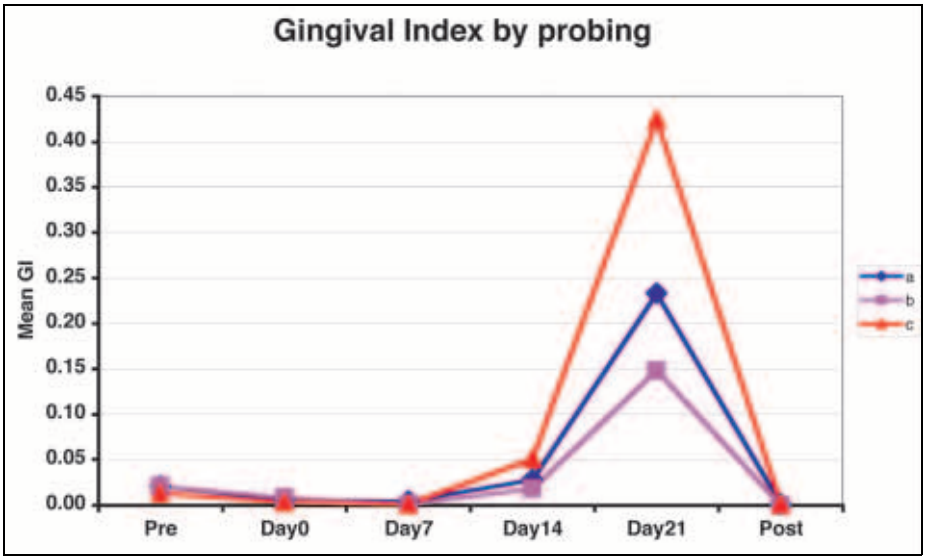

Fig 3 Gingival bleeding (GI = 2.3) for all treatment groups rinsing either with control(a), stannous fluoride (b), or the Triclosan/sodium fluoride (Colgate® Total) (c) at all time intervals. The stannous fluoride group is significantly lower ($p < 0.05$) than the Triclosan/sodium fluoride (Colgate® Total) (c) group.

gentle probing and excluded the application of visual criteria such as redness and swelling.

The experimental gingivitis model is an established standard for testing the antimicrobial activity of a mouthrinse or dentifrice slurry (Lang et al, 2002), thereby avoiding the influence of oral hygiene habits and hence allowing for undisturbed plaque development on originally plaque-free tooth surfaces. Thus, the effect of the host response to the biofilm formation may be evaluated in a reproducible manner and within a relatively short period of time. Within the course of 21 days of no-oral-hygiene practices, every volunteer predictably develops measurable gingivitis to various degrees and severity (Löe et al, 1965). As a positive control, chlorhexidine digluconate mouthrinses have often been used to inhibit the development of bacterial plaque and hence, completely avoid the development of gingivitis (Lang et al, 1986; Siegrist et al, 1986). However, even without the incorporation of a positive control into a clinical experiment, the model has been used for testing even the antimicrobial effect of compounds with low clinical efficacy (Lang et al, 2002).

In the present study, twice-daily rinses with 0.45% SnF2 significantly reduced the development of gingivitis in comparison to a commercially available Triclosan/sodium fluoride/copolymer containing dentifrice (Colgate® Total), although the effect on the biofilm development was minimal and did not differ from that of the two compounds for comparison. The inability of SnF2 to inhibit biofilm formation significantly is consistent with clinical results previously reported (Beiswanger et al, 1995). In that study, significant reductions in gingivitis were not accompanied by corresponding decreases in plaque scores.

Topical stannous fluorides have been used in dentistry since the 1950s (König, 1959), and only minor side effects have been reported. The major reported effects of stannous fluorides is its reduction of *Streptococcus mutans* levels in the oral cavity, hence a documented anticaries effect (for review see Tinanoff et al, 1985, 1989). Subsequently, the effects of stannous fluoride on plaque formation have been documented with 0.1% mouthrinse (Tinanoff et al, 1980) or 0.4% mouthrinse (Bay and Rölla, 1980; Øgaard et al, 1980; Yankell et al, 1982; Hefti and Huber, 1987). The effect of topical applications of 0.4% stannous fluoride to reduce gingival inflammation has also been documented in laboratory animals (Hock et al, 1979). Similar effects were demonstrated in humans using 0.4% stannous fluoride gels (Boyd et al, 1988; Wolff et al, 1989).

The results of this clinical trial demonstrated that, over a three-week period, the application of a 0.45% SnF2 gel significantly inhibited the onset of gingivitis compared to Triclosan/sodium fluoride/copolymer (*Colgate® Total*). After suspending oral hygiene practices, biofilm formation was seen in all three groups without any clinically measurable differences between the groups. Yet, the only effect in delaying the development of gingivitis was seen in the group rinsing with 0.45% stannous fluoride

twice daily. This suggests that a chemical agent may achieve a preventive effect for gingivitis development without the concomitant reduction in supragingival plaque biomass. The mechanisms for this phenomenon are not completely understood and may be discussed both in terms of changing virulence or a host response being affected by the drug. The lack of the plaque reduction observed in the present study may be related to the effects of SnF_2 in promoting the deposition of thick pellicle protein films on the tooth surface that visually may be confused with plaque biomass. Such thickening of pellicle has been reported on teeth following SnF_2 treatment (Tinanoff et al, 1979). During the attempt to explore possible mechanisms for the antibacterial effects of SnF_2, most authors focused on either alteration of the bacterial adhesion/cohesion (Tinanoff et al, 1976; Ota et al, 1989) or bacterial growth (Svatun et al, 1978; Lilienthal et al, 1956). On the basis of these considerations and with respect to the well-established anticaries effect (Svanberg et al, 1982; Faller et al, 1995), stannous fluoride appears to be a chemotherapeutic agent that should be further investigated for its preventive effects.

The present study demonstrated statistically significant differences between the gingivitis-delaying effect of stannous fluoride rinses and the Triclosan/sodium fluoride/copolymer-containing commercially available dentifrice slurries. However, neither dentifrices containing Triclosan/sodium fluoride/copolymer nor stannous fluoride possessed sufficient antimicrobial activity to suppress biofilm formation in the absence of normal oral hygiene.

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