Effect of NaF-, SnF_2-, and chlorhexidine-impregnated birch toothpicks on mutans streptococci and pH in approximal dental plaque

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The antimicrobial effect of birch toothpicks impregnated with 4% NaF, 8% SnF_2, or 2% chlorhexidine was studied both in vitro and in vivo. A non-impregnated toothpick served as a control. In vitro, suspensions of *Streptococcus mutans* were exposed to the various toothpicks for 20 min and then cultured on blood agar. The results of this susceptibility test revealed the following ranking order with respect to inhibition: chlorhexidine > SnF_2 > NaF and non-impregnated; with significant differences in colony-forming units (CFU) between these three groups. In vivo, 12 individuals used the 4 types of toothpick 3 times a day for 5 days in a procedure with a crossover design. Saliva and approximal plaque samples were collected at baseline and on various occasions up to 23 days after the treatment. At the same time, plaque-pH was measured at approximal sites 10 min after rinsing with 10% sucrose. The results of these in vivo experiments revealed lower proportions of mutans streptococci after using all four types of toothpick, but the reduction was significant only after 2 days for the toothpicks impregnated with SnF_2 and chlorhexidine ($P < 0.05$). On the sampling occasions 9 and 23 days after the treatment, the mutans streptococci were more or less back to baseline levels again. In saliva no significant differences in the number of mutans streptococci were found either within or between the four treatments. No significant differences were found regarding decline in the plaque-pH between the NaF-, SnF_2-, chlorhexidine-, and non-impregnated toothpicks on any of the sampling occasions. □ *Antimicrobials; dental plaque; fluoride; saliva; toothpicks*

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Recent studies have shown that birch and lime toothpicks impregnated with sodium fluoride (NaF) release a considerable amount of fluoride both in vitro (1, 2) and in vivo (3). The release from the toothpicks is rapid, as about 11 mM of fluoride was found in interproximal areas after the use of fluoridated toothpicks, representing 25% of the total amount of fluoride in the toothpicks (3). This may be of clinical importance in the prevention of dental caries, and this hypothesis is supported by a recent study showing that the use of NaF-impregnated toothpicks inhibits the demineralization and stimulates the remineralization of approximally located enamel and dentin specimens in vivo to a greater degree than non-impregnated toothpicks (4).

Since wooden toothpicks appear to be useful vehicles for the delivery of NaF to the approximal area, it may be of interest to test some other caries-reducing substances, such as stannous fluoride (SnF_2) and chlorhexidine (5, 6). The aim of the present study was therefore to evaluate the effect of birch toothpicks impregnated with NaF, SnF_2, or chlorhexidine on the prevalence of mutans streptococci and on the decline in pH in approximal dental plaque.

Materials and methods

Triangular birch toothpicks (TePe Munhygienprodukter, Malmö, Sweden) impregnated with 4% NaF (E Merck, Darmstadt, Germany), 8% SnF_2 (Apoteksbolaget, Göteborg, Sweden), or 2% chlorhexidine gluconate solution (Apoteksbolaget) and non-impregnated toothpicks (controls) were used. The toothpicks were impregnated for 30 min in our laboratory and were then kept at room temperature for 24 h for drying. The impregnation was carried out just before each experimental period.

In vitro experiments

A washed, overnight culture of *Streptococcus mutans* strain IB was inoculated into Jordan's broth and adjusted to a concentration equal to the turbidity of no. 1 MacFarland standard. The suspensions were then diluted 300 times to obtain a cell density of around 10^6 colony-forming units (CFU)/mL. Ten toothpicks of each type (NaF-, SnF_2-, chlorhexidine-, and non-impregnated) were placed individually in 2 mL of the bacterial suspension and shaken in order to release the substances from the toothpick. After exposure for 20 min at 37°C, 0.5 mL was cultured on blood agar plates and incubated for 24 h in an atmosphere of 5% CO_2 and 95% N_2. The number of colonies were counted, and the mean values for each group were calculated.

In vivo experiments

Subjects. Twelve subjects, 5 men and 7 women, aged 21–35 years (mean age, 26 years), were selected on the basis of having $>10^5$ CFU of mutans streptococci per mL of saliva. They had a mean DMFS value of 18.8 (range, 7–39), a normal buffer capacity (mean final pH, 5.2 ± 1.5), and a normal salivary secretion rate of paraffin-stimulated whole

saliva (mean, 1.9 ± 1.6 mL/min). None of the participants had any clinically or radiographically detectable frank carious lesions.

Experimental design. The subjects participated in four different experimental periods, each lasting 4 weeks (Fig. 1), using NaF-, SnF_2-, chlorhexidine-, and non-impregnated toothpicks. The study was performed in double-blind fashion and with a crossover design, and it was approved by the Ethics Committee at Göteborg University. The subjects were carefully instructed on how to use the toothpicks in the following way. After moistening the toothpick in saliva for a few seconds (to release the active ingredient), it was moved back and forth five times in each approximal area, starting at 15/16 and then moving stepwise toward the central incisors. The same procedure was used in the 2nd, 3rd, and 4th quadrants using a new toothpick for each quadrant. In this way 4 toothpicks were used 3 times a day during a period of five days ($n = 3 \times 4 \times 5 = 60$ toothpicks). Between the 4 periods there was a wash-out period of at least 6 weeks; this was considered to be sufficient to avoid a carryover effect. A low-fluoride dentifrice (Acta, 0.025% fluoride as NaF, Cederroth, Falun, Sweden) was used 4 days before and during each experimental period. No approximal tooth-cleaning was permitted during the 2 days prior to the first sampling occasion and throughout the whole test period (up to 23 days after the treatment; Fig. 1).

Saliva and plaque samples. Saliva and plaque samples were collected at baseline (day 1) and up to 23 days after the treatment (Fig. 1). No eating or toothbrushing was allowed for about 2 h and 4 h, respectively, before sampling. A paraffin-stimulated whole saliva sample was collected for 2 min, and 1 mL was transferred to a bottle containing 5.7 mL VMG II transport medium (7). Plaque samples were taken using sterile, unwaxed dental floss (Butler, John O. Butler Co., Chicago, Ill., USA) in the approximal spaces between the teeth 15/16 and 35/36. The dental flosses with plaque were cut off and placed in pre-reduced transport medium (8) with glass beads. The plaque samples were sonically dispersed for 10 s using a Branson sonifier (W185), serially diluted in 0.05 M phosphate buffer with 0.4% KCl (pH 7.1) and plated on mitis salivarius bacitracin (MSB) agar (9) for the growth of mutans streptococci and on blood agar for total colony counts. The saliva samples were dispersed on a Whirlimixer for 30 s, diluted, and plated on MSB agar. All MSB and blood agar plates were incubated in a gas mixture consisting of 95% N_2 and 5% CO_2 at 37°C for 2 days and 7 days, respectively. Mutans streptococci were identified by their characteristic colony morphology (10) and counted on the MSB agar plates. Their number in percent was determined in relation to the total count on the blood agar plates.

Plaque-pH determination. After the plaque sampling, the pH value was measured at the approximal sites of the contralateral teeth 25/26 and 45/46 (0-min value). After rinsing with 10 mL of a 10% sucrose solution for 1 min, the pH was measured 10 min later at the same sites (10-min value). The pH was determined using the microtouch method (11, 12) using an iridium microelectrode (diameter 0.1 mm, Beetrode MEPH-1, W.P. Instruments Inc., New Haven, Conn., USA) and a porous glass reference electrode (MERE 1, W.P. Instruments Inc.), both connected to a millivolt meter (Orion SA 720 pH/ISE Meter, Orion Research Inc., Boston, Mass., USA). A salt bridge was created by the subject dipping a finger in a 3 M KCl solution, also containing the reference electrode. The electrode was calibrated against a standard pH buffer at pH 7.0 immediately before and after each measurement.

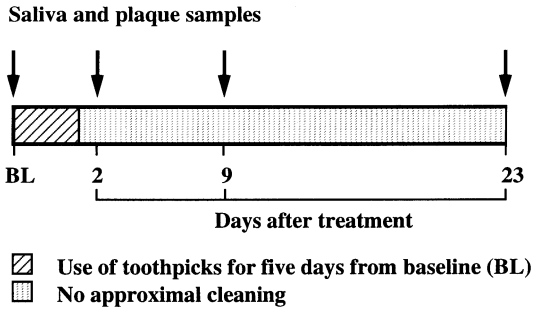

Fig. 1. Experimental design used in the study. The schedule was repeated 4 times with at least 6 weeks between periods.

Statistical analyses

The susceptibility values from the in vitro tests were compared using an analysis of variance (ANOVA) followed by Student Newman Keul's test. In the in vivo experiments, the mean of the two plaque samples for mutans streptococci and that of the two sites for plaque-pH measurements were used in the statistical analyses. The mutans streptococci means were logarithmically transformed in order to normalize their distribution. Comparisons between the four treatments were performed using ANOVA, followed by Student Newman Keul's test, and between the values before and after each test period using a paired *t* test (two-tailed). A *P* value of <0.05 was considered statistically significant.

Results

In vitro experiments

In the broth susceptibility tests, the NaF-impregnated toothpicks showed 14% lower counts of *S. mutans* when compared with the control toothpicks (Table 1). The corresponding values for SnF_2-impregnated toothpicks and for chlorhexidine-impregnated toothpicks were 73% and 99%, respectively. No antibacterial effect was obtained for the non-impregnated toothpicks. The effect exerted by the SnF_2- and chlorhexidine-impregnated toothpicks differed significantly from one another and from that of the NaF- and non-impregnated toothpicks ($P < 0.001$).

Table 1. Broth susceptibility testing of non-impregnated toothpicks (control) and toothpicks impregnated with a 4% NaF, 8% SnF_2, or 2% chlorhexidine solution. Mean ± standard deviation given*

	Streptococcus mutans	
Toothpick impregnation	CFU × 10^6	% inhibition
None (control)	1.09 ± 0.22	0
NaF	0.94 ± 0.32	14
SnF_2	0.30 ± 0.30	73
Chlorhexidine	0.01 ± 0.01	99

* Significant differences in colony-forming units (CFU) between types of toothpick ($P < 0.001$, ANOVA), except between the control and NaF toothpicks.

In vivo experiments

Lower proportions of mutans streptococci were found for all four types of toothpick compared with baseline (Fig. 2). Two days after the treatment, the reduction was statistically significant for toothpicks containing chlorhexidine and SnF_2 ($P < 0.05$). Between the four treatments, there were no statistically significant differences. However, the proportions of mutans streptococci after using NaF- and non-impregnated toothpicks were somewhat higher than after using SnF_2- and chlorhexidine-impregnated toothpicks. On the sampling occasions 9 and 23 days after the treatment, the mutans streptococci were back to baseline levels again for non-impregnated (control) and NaF-impregnated toothpicks, whereas the SnF_2 and chlorhexidine toothpicks displayed a tendency toward lower mean values than at baseline.

In saliva no significant differences in the number of mutans streptococci were found either between or within the four treatments. The mean salivary numbers ranged from 0.4 to 0.8 × 10^5 CFU/mL.

The mean plaque-pH values before (0-min value) and 10 min after the mouthrinse with sucrose are shown in Fig. 3. At baseline the pH dropped for all toothpick treatments to 5.8–5.9. Two days after the treatment period, the decline was reduced for all types of toothpick. However, the reduction was not significantly different from the baseline levels, and no differences were observed between the various toothpicks. Twenty-three days after treatment, the decline in pH was back to the baseline levels, but with a somewhat more pronounced decline in the interproximal plaque where the non-impregnated and the NaF-impregnated toothpicks had been used when compared with the interproximal plaque where the other two types of toothpick had been used.

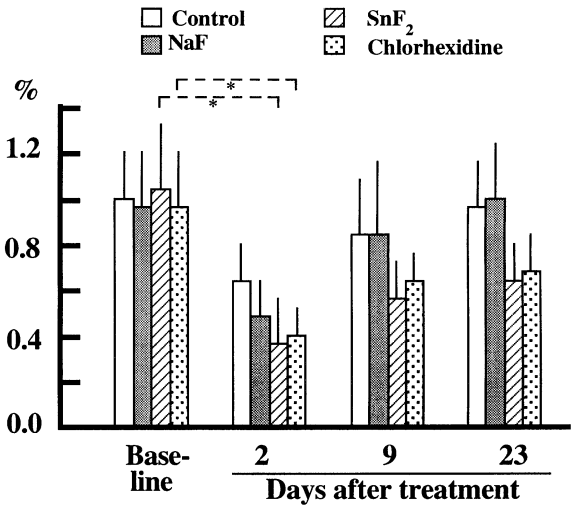

Fig. 2. Proportions of mutans streptococci of total counts (percent, mean ± standard error of the mean) at 15/16 and 35/36 before and after 5 days' use of non-impregnated toothpicks or toothpicks impregnated with 4% NaF, 8% SnF_2, or 2% chlorhexidine.

Discussion

The use of toothpicks for 5 days resulted in a decrease in the population of mutans streptococci in approximal plaque after all four treatments. The reduction was more pronounced when the toothpicks impregnated with antimicrobial agents (SnF_2 and chlorhexidine) were used, but the differences between the various toothpicks were not statistically significant. The SnF_2 and chlorhexidine toothpicks produced the lowest levels of microorganisms, which were also significantly lower than at their corresponding baseline levels. However, the inhibitory effect on the mutans streptococci in vivo was only transient. So, 9 days after the use of the toothpicks, the mutans streptococci had more or less returned to the baseline levels after using both the non-impregnated and the NaF-impregnated toothpicks. For the toothpicks impregnated with SnF_2 and chlorhexidine, the recolonization was somewhat slower, and a small remaining effect was still apparent after 3 weeks. The return of the microorganisms after the treatment is in line with the recolonization pattern in many studies that have examined the effect on mutans streptococci of various preventive measures (6).

Although there was a tendency toward decreased levels of mutans streptococci after treatment with the non-impregnated toothpicks, the reduction was not significant. This finding is in agreement with studies using other mechanical hygiene methods, such as flossing (13) and professional tooth-cleaning (14). The short-term reduction in the mutans streptococci counts may therefore be due to the mechanical removal of interdental microorganisms.

In comparison with the non-impregnated toothpicks, the additional inhibition in the population of mutans streptococci obtained after using the NaF toothpicks was only marginal and is in line with the results obtained in the in vitro experiments. So, in the broth susceptibility test, only a small, non-significant reduction in *S. mutans* growth was observed when the NaF toothpicks were used. According to Maltz & Emilson (15), around 75–150 mM of fluoride is required for a bactericidal effect on *S. mutans* in solutions. In approximal plaque a peak of 11 mM of fluoride was observed 2 min after using NaF toothpicks,

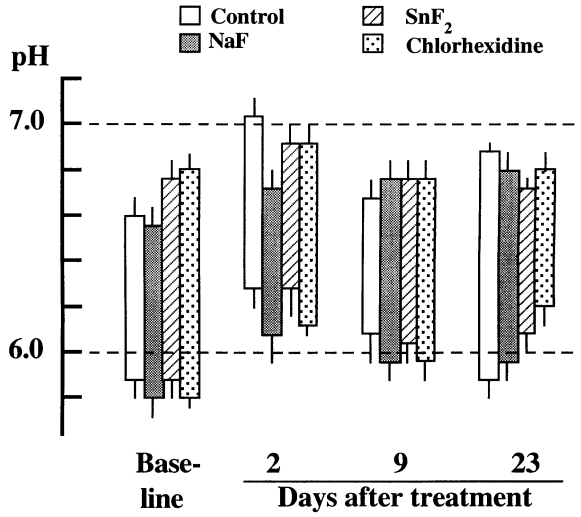

Fig. 3. Decline in pH in approximal plaque (mean ± standard error of the mean) 10 min after rinsing with a 10% sucrose solution for 1 min with 4 types of toothpick (non-impregnated control, NaF-, SnF_2-, and chlorhexidine-impregnated). The 0-min value is shown at the top of each column, and the 10-min value at the bottom. Declines in pH are given at baseline and 2, 9 and 23 days after the treatment.

with a rapid decrease thereafter (3). The amount of fluoride released from the toothpicks is thus much lower than the concentration required for a bactericidal effect, but it may nonetheless be of clinical importance for the prevention of dental caries. This is supported by the fact that treatment with NaF varnish produces a substantial reduction in caries (16), even if no significant effect on the plaque and salivary levels of *S. mutans* has been found (17). Moreover, when topically applied NaF gels are used in irradiated patients, only a marginal effect on the plaque levels of *S. mutans* compared with a fluoride-free gel has been reported (18). The fluoride concentration obtained by the NaF toothpicks, when used regularly, may therefore affect the de- and remineralization of enamel and dentin (19). This is supported by a recent study showing that demineralized pieces of dentin carried by subjects were remineralized after the use of NaF toothpicks for 4 weeks (4).

The somewhat better in vivo effect on mutans streptococci of toothpicks impregnated with SnF_2 compared with NaF is supported by the broth susceptibility tests, where a marked and more pronounced inhibition was observed with SnF_2 toothpicks compared with NaF ones. In other in vitro studies, it has been observed that SnF_2 is more bactericidal than NaF at the same fluoride concentration, and considerably lower concentrations are required for SnF_2 to suppress microorganisms (15, 20). The improved antimicrobial effect of SnF_2 could perhaps be ascribed to the metal component, which appears to play a major role in its bactericidal effect (21). An alternative method for bringing SnF_2 into approximal areas is to use a dental floss. Keene et al. (22) applied SnF_2 in this way and found a clear-cut effect on *S. mutans*. Moreover, when an 8% SnF_2 solution was applied with cotton pellets, a reduction in the number of *S. mutans* in approximal plaque was found (23).

The toothpicks containing chlorhexidine showed a pronounced initial effect on the approximal population of mutans streptococci. In addition, other modes of delivering chlorhexidine locally to approximal sites have been tested, such as using a syringe (24) or dental floss (23, 25). However, chlorhexidine used in these ways produced only transient reductions in the mutans streptococci counts, as in the present study. No significant changes in the proportion of mutans streptococci in saliva were noted for the chlorhexidine toothpicks or for any of the other types of impregnated toothpick. The reason may be that the effect on the microorganisms at the interproximal sites was not sufficient to affect the salivary microflora, which is composed of bacteria from all the surfaces in the oral cavity.

In the present study, plaque-pH was measured 10 min after the sucrose rinse, since it has been shown that the pH-curve reaches a minimum value after this time interval (12). There was, however, no significant difference in the plaque-pH response to sucrose in the interproximal spaces before and after the treatment for any of the toothpick groups. This may be due to the somewhat modest reduction in total plaque bacteria, reflected here by the population of mutans streptococci. There was, however, a tendency toward a lower fall in pH 2 days after the treatment for all 4 types of toothpick, which may have been caused by interference by the remaining approximal plaque bacteria.

To conclude, this study showed that toothpicks, especially those impregnated with SnF_2 and chlorhexidine, had an effect on the population of mutans streptococci and on the decline in pH in plaque, but that it was small and only of short duration.

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References

1. Petersson LG, Kashani H, Birkhed D. In vitro and in vivo studies of an NaF impregnated toothpick. Swed Dent J 1994;18:69–73.
2. Kashani H, Birkhed D, Petersson LG. Uptake and release of fluoride from birch and lime toothpicks. Eur J Oral Sci 1995; 103:112–5.
3. Kashani H, Birkhed D, Petersson LG. Fluoride concentration in the approximal areas after using toothpicks and other fluoride-containing products. Eur J Oral Sci 1998;106:564–70.
4. Kashani H, Birkhed D, Arends J, Ruben J, Petersson LG. Effect of toothpicks with or without fluoride on de- and remineralization of enamel and dentine in situ. Caries Res. In press 1998.
5. Klock B, Serling J, Kinder S, Manwell MA, Tinanoff N. Comparison of effect of SnF_2 and NaF mouthrinses on caries incidence, salivary *S. mutans* and gingivitis in high caries prevalent adults. Scand J Dent Res 1985;93:213–7.
6. Emilson CG. Potential efficacy of chlorhexidine against mutans

streptococci and human dental caries. J Dent Res 1994;73:682–91.
7. Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Odontol Tidskr 1966;74:1–380.
8. Syed SA, Loesche WJ. Survival of human dental plaque flora in various transport media. Appl Microbiol 1972;24:638–44.
9. Gold OG, Jordan HV, van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357–64.
10. Emilson CG. Prevalence of *Streptococcus mutans* with different colonial morphologies in human plaque and saliva. Scand J Dent Res 1983;91:26–32.
11. Scheie AA, Fejerskov O, Lingström P, Birkhed D, Manji F. Use of palladium touch microelectrodes under field conditions for in vivo assessment of dental plaque pH in children. Caries Res 1992;26:44–52.
12. Lingström P, Imfeld T, Birkhed D. Comparison of three different methods for measurement of plaque-pH in humans after consumption of soft bread and potato chips. J Dent Res 1993;72:865–70.
13. Axelsson P, Kristoffersson K, Karlsson R, Bratthall D. A 30-month longitudinal study of the effects of some oral hygiene measures on *Streptococcus mutans* and approximal dental caries. J Dent Res 1987;66:761–5.
14. Emilson CG, Axelsson P, Kallenberg L. Effect of mechanical and chemical plaque control measures on oral microflora in school-children. Community Dent Oral Epidemiol 1982;10: 111–6.
15. Maltz M, Emilson CG. Susceptibility of oral bacteria to various fluoride salts. J Dent Res 1982;6:786–90.
16. Koch G, Petersson LG. Caries preventive effect of a fluoride-containing varnish (Duraphat) after 1 year's study. Community Dent Oral Epidemiol 1975;3:262–6.
17. Zickert I, Emilson CG. Effect of a fluoride-containing varnish on *Streptococcus mutans* in plaque and saliva. Scand J Dent Res 1982; 90:423–8.
18. Brown LR, Dreizen S, Handler S. Effects of selected caries preventive regimes on microbial changes following irradiation induced xerostomia in cancer patients. Microbial Abstr 1976; 1:275–90.
19. ten Cate JM. In vitro studies on the effect of fluoride on de- and remineralisation. J Dent Res 1990;69:614–9.
20. Mayhew RR, Brown LR. Comparative effect of SnF_2, NaF, and $SnCl_2$ on the growth of *Streptococcus mutans*. J Dent Res 1981; 60:1809–14.
21. Oppermann RV, Johansen JR. The effect of fluoride and non-fluoride salts of copper, silver and tin on the acidogenicity of dental plaque in vivo. Scand J Dent Res 1980;88:476–80.
22. Keene HJ, Shklair IL, Mickel GJ. Effect of multiple dental floss-SnF_2 treatment on *Streptococcus mutans* in interproximal plaque. J Dent Res 1977;56:21–7.
23. Schaeken MJM, DeJong MH, Franken HCM, Van der Hoeven JS. Effects of highly concentrated stannous fluoride and chlorhexidine regimes on human dental plaque flora. J Dent Res 1986;65:57–61.
24. Kristoffersson K, Bratthall D. Transient reduction of *Streptococcus mutans* interdentally by chlorhexidine gel. Scand J Dent Res 1982;90:417–22.
25. Gisselsson H, Birkhed D, Björn AL. Effect of professional flossing with chlorhexidine gel on approximal caries in 12- to 15-year-old schoolchildren. Caries Res 1988;22:187–92..

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