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# Effect of Fluoridated Toothpicks and Dental Flosses on Enamel and Dentine and on Plaque Composition in situ 

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## Key Words

Approximal caries • Dental floss • Dentine • Enamel • Fluoride • Toothpick, fluoridated


#### Abstract

The aim was to evaluate the effect of two toothpicks and two dental flosses on demineralized enamel and dentine and on plaque composition, using an in situ model with simulated approximal spaces within dentures. Fifteen subjects with full dentures were recruited and 14 completed the study. It consisted of two crossover periods, the first comparing a birch toothpick with AmF and a birch toothpick with NaF , and the second comparing a dental floss with $\mathrm{AmF}+\mathrm{NaF}$ and a dental floss without F . Between these four periods, there was a control period without any approximal cleaning. In small, rectangular sample holders, one enamel and one dentine specimen were embedded, forming a triangular, approximal-like space. Two sample holders were mounted in the premo-lar-molar region of the upper or lower dentures. The subjects used the toothpicks or dental flosses 3 times/day for 4 weeks. The results showed that all toothpicks and dental flosses inhibited further demineralization compared to the control period ( $p<0.001$ ). The dental flosses were somewhat more effective in this respect than the toothpicks, especially in dentine. There were relatively small numerical differences between AmF and NaF toothpicks,


but in favour of NaF regarding mineral gain ( $\mathrm{p}<0.05$ ). The fluoridated floss gave somewhat less lesion depth in dentine than the non-fluoridated floss ( $p<0.01$ ). Toothpicks and flosses resulted in lower counts of microorganisms in plaque compared to the control period ( $p<$ 0.001); the AmF toothpick gave a more pronounced reduction than the NaF toothpick ( $\mathrm{p}<0.001$ ).

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Dental caries mainly affects the approximal smooth surfaces and the occlusal fissures [Seppä et al., 1991]. While the buccal and lingual surfaces benefit from fluoride ( F )-containing products, such as dentifrices and tablets, the effect on the approximal surfaces appears to be less pronounced [Granath et al., 1978; Li et al., 1994; Øgaard et al., 1994]. This suggests that targeted topical F application is essential to these less accessible areas of the dentition. One possibility to apply F into the approximal area is to use fluoridated toothpicks and dental flosses. Several brands are now available on the market and have recently been evaluated in our laboratory [Kashani et al., 1995; 1998b; Särner et al., 2003].

Toothpicks impregnated with sodium fluoride ( NaF ) have been evaluated in an experimental model in situ [Kashani et al., 1998a]. The results showed that 4 weeks' daily use of toothpicks, especially of F-impregnated toothpicks, reduces the demineralization of enamel and den-

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tine at approximal sites. In vitro and in vivo studies have shown that the uptake of $F$ into enamel increases from dental floss impregnated or soaked with F [Bohrer et al., 1983; Jørgensen et al., 1989; Modesto et al., 1997].

Beside NaF, amine fluoride (AmF) is also used for impregnation of toothpicks and flosses. This F compound has been used as an active ingredient in toothpaste for more than 30 years [Mühlemann et al., 1957; Marthaler, 1968; Cahen et al., 1982]. Its caries-preventive effect is considered to be equivalent or superior to NaF [Warrick et al., 1999]. The effect of AmF is not only due to the F anion, but also to its surface and antibacterial activity [Nemes et al., 1991]. However, to our knowledge there has been no comparison of toothpicks and dental flosses impregnated with NaF and AmF. The aim of the present study was to evaluate the effect of two toothpicks and two dental flosses on demineralized enamel and dentine and on plaque composition, using an in situ model with simulated spaces within dentures.

## Material and Methods

## Subjects

Fifteen subjects with full dentures, 9 men and 6 women, with a mean age of 65 years (range 45-82 years), were included in the study. They were all patients at the Public Dental Clinic in Mölnlycke, Sweden. The study was approved by the Ethics Committee at Sahlgrenska Academy at Göteborg University and informed consent was obtained prior to the study.

## Toothpicks and Dental Flosses

The following four products were tested: (1) a birch toothpick with AmF (called AmF toothpick); (2) a birch toothpick with NaF (called NaF toothpick); (3) a dental floss with a mixture of NaF and AmF (called NaF/AmF floss), and (4) a dental floss without fluoride (called F-free floss). Products No. 1, 3 and 4 were manufactured by GABA International AG, Münchenstein, Switzerland and No. 2 by TePe Munhygienprodukter AB, Malmö, Sweden.

## Enamel and Dentine

Enamel and dentine specimens were prepared from extracted, sound human premolars and molars. Both the enamel and dentine samples were demineralized for 10 days in a $6 \% \mathrm{CMC}$ gel containing 0.1 M lactic acid (titrated to pH 5 with 10 M KOH ), resulting in subsurface lesions of around $100 \mu \mathrm{~m}$. More than 500 demineralized specimens were prepared for the study and approximately 200 of these were used to provide starting values of the initial lesion depth and mineral loss.

## Sample Holder

One demineralized enamel and one demineralized dentine specimen were embedded in self-curing polymethylmethacrylate (Candulor Autoplast, Wangen, Switzerland) in a small, rectangular sample holder (Evergreen scale models, Kirkland, Wash., USA), measuring $9.5 \times 6.3 \times 5.0 \mathrm{~mm}$ (length $\times$ height $\times$ depth). The specimens were
positioned in the holder forming a triangular, approximal-like space (fig. 1A, B). Two sample holders were mounted in the premolar-molar region of the denture either in the upper or lower jaw (depending on available space in the denture), one on the left and one on the right side (fig. 1C). They were mounted in such a way that a toothpick or a dental floss could be used for approximal cleaning (fig. 1D). Only one holder could be mounted in 6 of the 15 patients due to lack of space.

## Treatment

All subjects participated in five test periods altogether (two toothpick periods, two dental floss periods and one control period), each lasting for 4 weeks. The study was carried out double-blind and with a crossover design with respect to the two toothpicks and the two dental flosses. The two toothpick periods were carried out first (called periods 1 and 2 ), followed by a 4 -week period without any approximal cleaning (called control period or period 3). Finally, the two dental floss periods were performed (called periods 4 and 5). This means that the total experimental period was $5 \times 4=20$ weeks.

The participants were carefully instructed and trained in how to use the toothpicks and dental flosses by one of the investigators (B.S.) before the study started. The cleaning procedure was always carried out with the dentures in place in the mouth. A fresh toothpick or a $20-\mathrm{cm}$-long piece of dental floss was used for half a minute for each sample holder. The patients were instructed to pay attention to both the mesially and distally oriented specimens in each sample holder. The approximal cleaning was carried out 3 times a day (after breakfast, after lunch and before bedtime), except during the 4 -week control period. An F-free toothpaste (BlåVitt, Konsum, Stockholm, Sweden), a soft toothbrush ( TePe ) and tap water were used for cleaning the dentures (except the area where the sample holders were located). This was carried out twice a day in the hand basin, in the morning and in the evening, just before using the toothpicks and dental flosses. Otherwise, the dentures were worn day and night, including the meals.

After each 4-week period, the sample holders were removed and replaced with new holders for the next 4-week period. The specimens were kept in a plastic jar, containing wet cotton rolls and sent to the Department of Dentistry and Dental Hygiene, University of Groningen, The Netherlands, where they were analysed by transversal microradiography (TMR).

## Transversal Microradiography

In the laboratory, the enamel and dentine specimens were first cleaned with a multi-tufted toothbrush under running tap water for half a minute to remove plaque. Subsequently, two sections were cut from the central part of each specimen ( $\sim 150 \mu \mathrm{~m}$ thick for enamel and $\sim 350 \mu \mathrm{~m}$ for dentine). These sections were ground down together with the control samples to around $80 \mu \mathrm{~m}$ for enamel and $140 \mu \mathrm{~m}$ for dentine and then microradiographed [Dijkman et al., 1986; Øgaard et al., 1986; Arends and ten Bosch, 1992]. Densitometric scanning of the microradiographs was carried out using computer-assisted video densitometry [Inaba et al., 1997]. Three scans $(400 \times 300 \mu \mathrm{~m})$ were made of each enamel and dentine microradiograph and the average was used for further analysis. Two parameters were assessed: (1) lesion depth $(\mu \mathrm{m})$ and (2) mineral loss ( $\Delta \mathrm{Z}$, in $\mathrm{vol} \% \times \mu \mathrm{m}$ ).

## Microbiology

After each test period, plaque samples from the experimental approximal sites were collected using a sterile, triangular-shaped Ffree birch toothpick. The tip of the toothpick with plaque was cut off and transferred to a bottle with prereduced transport medium [Syed


Fig. 1. The experimental model used in the study. A Sample holder $(9.5 \times 6.3 \times 5.0 \mathrm{~mm}$; length $\times$ height $\times$ depth $)$ with two embedded demineralized specimens, one enamel and one dentine. The specimens were mounted in the holder in acrylic, forming a triangular, approximal space. B Outline of the sample holder. C Denture where one holder has been mounted in the molar region of an upper denture. D Toothpick introduced into the approximal area.
and Loesche, 1972] and glass beads. The plaque samples were sonically dispersed for 10 s and serially diluted in 0.05 M phosphate buffer with $0.4 \% \mathrm{KCl}(\mathrm{pH} 7.1)$ and plated on four different media: (1) blood agar for total count; (2) CFAT agar [Zylber and Jordan, 1982] for actinomycetes; (3) mitis salivarius bicitracin agar (MSB) [Gold et al., 1973] for mutans streptococci, and (4) Rogosa SL agar (Difco Laboratories, Detroit, Mich., USA) for lactobacilli. The blood and CFAT agar plates were incubated in a gas mixture of $95 \% \mathrm{~N}_{2}$ and $5 \%$ $\mathrm{H}_{2}$ at $37^{\circ} \mathrm{C}$ for 7 and 4 days, respectively. The MSB agar plates were incubated in a candle jar at $37^{\circ} \mathrm{C}$ for 2 days. The Rogosa SL agar plates were grown in air at $37^{\circ} \mathrm{C}$ for 3 days. Colonies with characteristic morphologies for mutans streptococci and actinomycetes were counted on MSB agar and CFAT agar, respectively. On blood agar and Rogosa SL agar, all colonies were counted.

## Statistical Analysis

The mean values for the specimens on the left and right side of the mouth (for the subjects where two samples were inserted) were calculated for each individual and each treatment. For the microbiological data, all values were transferred to logarithms. Student's $t$ test was used to compare the group results for the TMR data with the initial (starting) values. A linear mixed statistical model [Pinheiro and Bates, 2000] was used to compare effects of the two toothpicks and of
the two flosses, taking into account possible differences between periods. The model included a random patient effect and fixed effects due to treatment and period. This analysis was carried out in S-Plus (version 6.1; Insightful Corporation, Seattle, Wash., USA), separately for each of eight variables (lesion depth and $\Delta \mathbf{Z}$ for dentine and enamel and CFU for the four microbiological counts). Results are presented as estimated differences, p values and $95 \%$ confidence intervals. Presented $p$ values were not adjusted for multiple testing. To account for multiple tests (separately for the four TMR variables and the four microbiological variables), the results were considered significant at the $5 \%$ level when the unadjusted p values did not exceed $1 \%$. The effects of the two toothpicks compared to the control period on the one hand and the two flosses compared to the control period on the other hand were also analysed using the same statistical method.

## Results

Of the 15 subjects, 1 dropped out from the study after the first toothpick period due to illness; the other 14 participated in all five periods. Five sample holders came


Fig. 2. Box plots of lesion depth and mineral loss for all five runs compared to the initial demineralization (drawn horizontal lines) for enamel (left) and dentine samples (right). The line indicates the median, the box the 25 th and 75 th percentiles, and the whiskers the 10th and 90th percentiles. The mean is shown as a grey square for
loose from the dentures, a majority of them during period 1. Because of abrasion of enamel caused by the toothpicks, 3 samples could not be analysed and another 8 were microradiographed but subsequently considered to be too abraded to be included in the statistical analysis. Thus, when evaluating the TMR data for enamel from the two toothpick periods, only the non-abraded samples were used in the statistical analyses ( 30 samples in 12 patients). No abrasion was detected in dentine after using the toothpicks or in enamel and dentine after using the dental flosses. This was confirmed by light microscopy. Thus, for all other analyses data from 14 patients were available.

The TMR data are shown in figure 2 and the average lesion profiles for all groups in enamel and dentine in figure 3 . The initial demineralization values in enamel were $106 \pm 10.7 \mu \mathrm{~m}$ for lesion depth and $5,588 \pm 938 \mathrm{vol} \% \times$ $\mu \mathrm{m}$ for $\Delta \mathrm{Z}$. In dentine, the corresponding values were 107 $\pm 17.5 \mu \mathrm{~m}$ and $3,558 \pm 657 \mathrm{vol} \% \times \mu \mathrm{m}$, respectively.
extra information. Significant differences between the two toothpicks or two flosses are indicated by an asterisk. Differences between treatment groups (control, toothpicks or floss) are indicated using letters. Groups with the same letter are not significantly different.

Both the NaF and AmF toothpicks showed mineral gain when compared to the initial demineralization values. There was a lesion depth reduction for the enamel, but a lesion depth increase for the dentine samples ( $\mathrm{p}<$ 0.01 ). The control period resulted both in mineral loss and in lesion depth increase for the dentine ( $p<0.01$ ), but no significant changes for the enamel samples. The two dental flosses also resulted in remineralization compared to the initial demineralized samples regarding both lesion depth and mineral loss ( $\mathrm{p}<0.01$ ).

The results for the comparisons between toothpicks and flosses are summarized in tables 1 and 2 , respectively. When comparing the two toothpicks with each other, the NaF toothpick reduced the lesion depth in enamel more than the AmF toothpick (mean difference $30.6 \mu \mathrm{~m} ; \mathrm{p}=$ 0.004 ). The mineral loss difference in enamel was of borderline significance (mean difference $1,460 \mathrm{vol} \% \times \mu \mathrm{m}$; $\mathrm{p}=0.011$ ). The results for dentine showed the same trend,


Fig. 3. Average mineral loss profiles in enamel (left) and dentine (right) for the two toothpicks, the two flosses and the control. The mineral loss profile of the initial demineralization is also shown for reference.

Table 1. Statistical analysis of differences between the NaF toothpicks and the AmF toothpicks

| Variable | Difference <br> AmF toothpick - <br> NaF toothpick | p value | 95\% CI |
| :---: | :---: | :---: | :---: |
| Enamel |  |  |  |
| Lesion depth, $\mu \mathrm{m}$ | 30.6 | 0.004 | 12.7-48.8 |
| $\Delta \mathrm{Z}, \mathrm{vol} \% \times \mu \mathrm{m}$ | 1,460 | 0.011 | 441-2,479 |
| Dentine |  |  |  |
| Lesion depth, $\mu \mathrm{m}$ | 7.0 | 0.29 | -5.8-19.8 |
| $\Delta \mathrm{Z}, \operatorname{vol} \% \times \mu \mathrm{m}$ | 401 | 0.04 | 34-768 |
|  | Estimated ratio <br> AmF toothpick: <br> NaF toothpick | p value | 95\% CI |
| Total count | 0.13 | <0.001 | 0.06-0.29 |
| Actinomycetes | 0.09 | <0.001 | 0.03-0.29 |
| Mutans streptococci | 0.14 | <0.001 | 0.06-0.33 |
| Lactobacilli | 0.05 | <0.001 | 0.02-0.16 |

For the bacterial data, the differences between log-transformed data have been transformed back to geometric means, and the ratio of the means is given.

Table 2. Statistical analysis of differences between the F-free floss and the floss containing $\mathrm{NaF}+\mathrm{AmF}$

| Variable | Difference <br> F-free floss $\mathrm{NaF}+\mathrm{AmF}$ floss | p value | 95\% CI |
| :---: | :---: | :---: | :---: |
| Enamel |  |  |  |
| Lesion depth, $\mu \mathrm{m}$ | 1.0 | 0.89 | -13-15 |
| $\Delta \mathrm{Z}, \operatorname{vol} \% \times \mu \mathrm{m}$ | 559 | 0.13 | -143-1,261 |
| Dentine |  |  |  |
| Lesion depth, $\mu \mathrm{m}$ | 31 | 0.007 | 9.7-52 |
| $\Delta \mathrm{Z}, \operatorname{vol} \% \times \mu \mathrm{m}$ | 470 | 0.08 | -43-984 |
|  | Estimated ratio <br> F-free floss: <br> $\mathrm{NaF}+\mathrm{AmF}$ floss | p value | $95 \%$ CI |
| Total count | 1.73 | 0.12 | 0.88-3.40 |
| Actinomycetes | 1.87 | 0.20 | 0.73-4.8 |
| Mutans streptococci | 7.01 | 0.007 | 1.89-26 |
| Lactobacilli | 0.75 | 0.65 | 0.21-2.6 |

For the microbiological data, the differences between log-transformed data have been transformed back to geometric means, and the ratio of the means are given.

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Fig. 4. Box plots of the log-transformed number of CFUs of total count, actinomycetes, mutans streptococci, and lactobacilli for all groups. The line indicates the median, the box the 25 th and 75 th percentiles, and the whiskers the 10th and 90th percentiles. The mean is shown as a grey square for extra information. Significant
differences between the two toothpicks or two flosses are indicated by an asterisk. Differences between treatment groups (control, toothpicks or floss) are indicated using letters. Groups with the same letter are not significantly different.
but there was no significant difference. In the toothpick analysis, a significant effect $(p=0.001)$ of the period was found, with period 2 resulting in higher lesion depth (enamel and dentine) and mineral loss (dentine) values. When comparing the two dental flosses with each other, the floss containing $\mathrm{NaF}+\mathrm{AmF}$ showed a trend for less lesion depth and mineral loss for enamel than the F-free floss. The difference was statistically significant for lesion depth in dentine (mean difference $31 \mu \mathrm{~m} ; \mathrm{p}=0.007$ ). No 'period effect' was found for the flosses.

Box plots of the log-transformed values of the number of colony-forming units (CFU) in plaque for total count, actinomycetes, mutans streptococci and lactobacilli are shown in figure 4 . The comparison between toothpicks and flosses for the microbiological variables can also be seen in tables 1 and 2. For all four bacterial parameters, the AmF toothpick showed lower CFUs than the NaF toothpick. Here again, a significant influence of the peri-
od was found, where period 2 showed lower total count and lactobacilli than period 1 . There was a general trend that the control period resulted in the highest and the AmF toothpick period in the lowest counts for all types of microorganisms (fig. 4). The only significant difference for the flosses was for mutans streptococci, where the Ffree floss showed higher CFUs ( $\mathrm{p}=0.007$ ).

The overall comparison between the three treatments toothpicks (combined), control (no treatment) and floss (combined) - showed that for lesion depth and $\Delta \mathrm{Z}$ in dentine all three treatments were significantly different from each other; they increased in the order: floss $<$ toothpick $<$ control. For lesion depth and $\Delta \mathrm{Z}$ in enamel, the order was: toothpick $=$ floss $<$ control. For the microbiological variables, no overall difference between the toothpicks and flosses could be seen; all four periods showed, however, lower CFUs than the control group for total count and mutans streptococci.

## Discussion

The main finding of the TMR data was that toothpicks and dental flosses had a similar effect on enamel and dentine. Thus, while the caries lesions during the control period continued to grow (for dentine) or remained stable (for enamel), remineralization occurred during the test periods. The pattern was the same for lesion depth and for mineral loss, with the exception of lesion depth in dentine for the toothpicks, which increased. However, remineralization still occurred, and the mineral profiles (fig. 3) show that redistribution of the mineral has taken place, with a less acute lesion front. These results go hand in hand with the microbiological data, which showed that most types of microorganisms decreased during the toothpick and floss periods compared to the control period.

When comparing our study with Kashani et al. [1998a], who used a similar in situ experimental model for studying toothpicks as we did, the results look very much the same. Thus, it was found that a 4 -week period with daily use of toothpicks, especially with F-impregnated toothpicks, reduced the demineralization of enamel and dentine at the approximal sites. The reason why no remineralization was found in the study by Kashani et al. [1998a] may be that the approximal spaces were not so wide open and that the contact surface between the toothpicks and the sample holder probably was somewhat smaller.

It may be argued that a mouth with a complete denture is not comparable in every respect to an intact dentition. However, when comparing the data from two previous studies, we have found similar salivary F levels after toothbrushing in dentate and edentulous individuals wearing complete dentures [Sjögren and Birkhed, 1994; Sjögren et al., 1995]. No measurement of the F concentration in saliva was made in the present study, but recent studies in our laboratory have shown that the same three F products give very high F concentration in the approximal area in a natural dentition [Särner et al., 2003]. It is possible that the experimentally constructed approximal spaces, which were fairly wide open, resulted in the somewhat faster clearance of F interproximally than would have been the case if there had been closer contact between the teeth (as there is in a natural dentition). Another factor that may affect the approximal F concentration is the treatment time and treatment frequency of the toothpicks and flosses. Half a minute per site and 3 times/day may be considered higher than normal exposure.

One important aspect in a study like this is the compliance of the subjects. All individuals (who were carefully
recruited for the study) gave an assurance that they had followed the given instructions and that there was no difficulty involved in using the toothpicks and flosses in the two artificially constructed approximal areas with the dentures in place. This was confirmed by frequent personal contact between the investigator and the participants. We therefore believe that the model used in the present study is suitable and reliable for evaluating the effect of toothpicks and flosses on dental hard tissues.

The set-up of the study was mainly designed for the comparison of the two toothpicks and of the two flosses. Consequently, the crossover design was limited to those two groups. The patients and time period of all five periods were however the same, which may allow us to make some comparison among the various treatments. On the other hand, the total experimental period was rather long ( 20 weeks), which may have resulted in non-random differences between the periods, especially when comparing the toothpicks and flosses, which were separated by a control period. In the statistical model it was found that toothpick period 2 showed higher lesion depth and mineral loss for both dentine and enamel than period 1 . Such a 'period effect' was not observed in the floss study. Although results about the relative effects of toothpicks and flosses must be viewed with caution, the comparison with the control period seems more reliable, considering the large and opposite effects of the control period.

The toothpick study was compromised by the wear of the demineralized enamel samples. However, the differences show the same trend, whether the questionable samples were included or not, and also the same trend as for the dentine samples. We therefore believe that the data in the toothpick study are reliable. It is not clear if this enamel lesion wear is a clinically relevant phenomenon. There are some few reports in the literature indicating that extreme interproximal cleaning with toothpicks and dental flosses may cause abrasion of the tooth surface [Frayer, 1991; Gow and Kelleher, 2003]. The lesions in this study could be considered surface-softened lesions, with no clear surface layer, and low mineral content of the superficial enamel. This will have made the enamel lesions more susceptible to wear than would normally be the case.

The results of the floss study are somewhat surprising. Although the trend was that the fluoridated floss resulted in more pronounced reduction of lesion depth and mineral loss than the toothpicks, this was only significant for dentine. The effect of non-professional flossing on approximal caries has also given varying results [Granath et al., 1979; Wright et al., 1979; Gisselsson et al., 1983]. The present experimental study indicates that both non-fluoridated
and fluoridated flosses have a positive effect, especially on dentine. The reason why the flosses appeared to perform better regarding the TMR data than the toothpicks is not known. It can hardly be attributed to the bacterial effects, since there were only very small differences in the bacterial counts between the floss and toothpick periods. One reason may be that the subjects became more skilled in approximal cleaning since the two floss periods were carried out after the two toothpick periods. As already pointed out, it must be kept in mind that the comparison between toothpicks and flosses may have been confounded by some difference between the periods.

To conclude, this study showed that toothpicks and dental flosses have a positive effect on enamel and den-
tine in the approximal area. The NaF toothpick performed somewhat better than the AmF toothpick. Further research on the relative effectiveness of fluoridated toothpicks, dental flosses and other interdental cleaning aids should, however, be performed before any firm conclusion can be drawn regarding their clinical effect.

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