Inhibition of Acidogenicity in Dental Plaque by Sodium Fluoride Solution after Sucrose Rinse

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Objective: *To evaluate the effect of sodium fluoride rinse on sucrose-induced decrease in plaque pH with interdental plaque pH telemetry.*

Methods: *Six participants wearing partial lower prostheses incorporating a miniature glass pH electrode were included. The measurement of plaque pH was performed after 6 days of plaque accumulation. Three experiments were performed to test the effects of fluoride administered before (experiment I) or 10 min after (experiment II) and 25 min after (experiment III) a sucrose rinse. In experiment I, the subjects rinsed with 0.05% NaF solution before rinsing with 10% sucrose to examine the effect of fluoride on the sucrose-induced pH drop. In experiment II, the subjects first rinsed with 10% sucrose and then followed no subsequent rinsing or rinsing with tap water 10 min after sucrose expectoration, or 0.05%, 0.02% or 0.01% NaF solution for 2 min. In experiment III, the subjects first rinsed with 10% sucrose and then 25 min after sucrose expectoration, they rinsed with 0.05% NaF for 2 min. The plaque pH was continuously recorded for about 120 min.*

Results: *Without any subsequent rinses, the plaque pH decreased at 10 min to 4.36 and stayed below the critical pH 5.7 for about 85 min after the sucrose rinse. Subsequent water rinses showed little effect on the sucrose-induced decrease in plaque pH. Pretreatment of 0.05% NaF solution showed no effect on the subsequent sucrose-induced pH drop. Subsequent rinses with NaF solutions at 10 min after the sucrose rinse inhibited the sucrose-induced pH drop in a dose-dependent manner. Subsequent rinses with NaF solutions after the sucrose rinse also significantly reduced the time below pH 5.7 and the area of plaque pH curve under 5.7 ($AUC_{5.7}$). Furthermore, the $AUC_{5.7}$ value (3.99) of 0.05% NaF rinse at 10 min after the sucrose rinse was much smaller than that (57.01) of 0.05% NaF rinse at 25 min after the sucrose rinse.*

Conclusion: *Rinsing with 0.05% fluoride after carbohydrate consumption effectively reduced the acidogenicity of the plaque and could enhance the anticaries functions of fluoride.*

Key words: *fluoride, plaque pH, caries prevention, sucrose, Stephan curve*

Dental caries is a bacterial-based disease. Fluoride is known as the most effective anticaries agent currently available. Although the main mechanisms by which fluoride prevents dental caries are the inhibition of demineralisation and the enhancement of remineralisation of early caries lesions[1,2], the antimicrobial actions of fluoride contributing to its anticaries properties remains contentious[3]. Fluoride can inhibit the acid production of cariogenic bacteria in planktonic cells or biofilm model *in vitro*[4-10], and can even inhibit the acid production of cariogenic bacteria at the levels of fluoride in plaque[11]. However, the effectiveness of the fluoride rinse on acid production *in vivo* remains controversial. Pretreatment with 12 mM NaF (0.05% or 250 ppm)[12,13] or with 225 ppm or 900 ppm fluoride solution[14-16] results in no or small effects on plaque acidogenicity. The question remains as to whether the effects of fluoride on acidogenicity alone could sufficiently contribute to its anticaries properties *in vivo*.

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Caries is site specific. The controversial results of previous studies may be due to employing pooled supragingival plaque samples. In addition, the need for the appropriate methods used for evaluating the biological effects of fluoride *in vivo* was also suggested for these studies[3].With regard to the caries susceptible site of interproximal dental plaque, measurement of pH with pH telemetry could just adequately serve the purpose of evaluating the effects of fluoride on the acidogenicity of plaque, as this method can continuously record pH in undisturbed plaque[17-19]. Therefore, the purpose of the present study was to investigate the effects of a single rinse of fluoride solution on the acidogenicity potential of interproximal dental plaque before or after a sucrose rinse through pH telemetry.

Materials and methods

Subjects

Ethical approval was obtained from the Ethical Committee of Peking University Health Science Centre. Four men and two women (aged 56 to 72 yrs) were recruited from the Department of Prosthodontics, Peking University School and Hospital of Stomatology. Subjects with two or three missing teeth in the premolar and molar regions of the mandible were included, and they had no active caries lesions, unfilled cavities, periodontal disease, or other oral diseases. All subjects had a stimulated salivary secretion rate > 0.6 ml/min, the mean pH was 7.67, and informed consent was obtained.

Interdental plaque pH telemetry

The pH telemetry system was established and performed as described in detail previously[18,20]. Briefly, for each subject a mandibular partial prosthesis was fabricated incorporating a miniature glass pH electrode (W. Möller). The tip of the electrode faced the interdental surface of the subject's adjacent abutment teeth below the proximal contact point. The pH was continuously recorded (μR 1000, Yokogawa) and the original pH curve was scanned (intuos3, Vacom) and analysed by the computer software (TelDat, Version 1.5, Boling). The electrode was calibrated with a standard buffer of pH 7.00 before each test session.

Plaque accumulation

The accumulation of plaque on the tip of the electrode was performed as described in detail previously[18,20]. Briefly, the subjects were instructed to wear the prostheses with the clean electrodes remaining in place, and not to remove the dental device and not to alter their eating habits. They were asked to refrain from all oral hygiene measures for the entire experimental period, except for rinsing with water and tooth brushing without toothpastes. The pH measurement was performed in the early morning on the sixth day of plaque accumulation without having eaten or drunk anything except water before the test.

Experiment procedure

The experiment procedure was also performed as described in detail previously[18,20], but with minor modifications. The subjects were arranged to test one of the solutions each visit, with an interval of one week. Each subject attended the experiment on seven occasions in total.

Three experiments were performed to test the effects on plaque acidogenicity of fluoride administered before (experiment I) or 10 min after (experiment II) or 25 min after (experiment III) the sucrose rinse. A sucrose rinse without any treatment was initially administered as a control for each subject.

In experiment I, the effect of fluoride administered before the sucrose rinse on the plaque pH was tested. The experiment started with the chewing of paraffin for 3 min for plaque-pH normalisation. After an initial period of 20 min to establish the baseline value, the subjects rinsed with 15 ml 0.05% NaF for 2 min; 10 min after expectation, the subjects rinsed with 15 ml 10% sucrose for 2 min, and followed a further continuously plaque pH recording for about 80 min for each test.

In experiment II, the effect of fluoride administrated at 10 min after sucrose rinse on the plaque pH was examined. After an initial period of 20 min to establish the baseline value, the subjects rinsed with 15 ml of 10% sucrose for 2 min; 10 min after expectoration, the subjects rinsed with 15 ml of tap water or 0.05% or 0.02% or 0.01% NaF solution for 2 min at each visit, and followed a further continuously plaque pH recording for about 80 min for each test.

In experiment III, the effect of 0.05% NaF administrated at 25 min after sucrose rinse on the plaque pH, by when the most of the sucrose in the plaque is metabolized[21], was examined. After an initial period of 20 min to establish the baseline value, the subjects rinsed with 15 ml of 10% sucrose for 2 min; 25 min after expectoration, the subjects rinsed with 15 ml of 0.05% NaF solution for 2 min, and followed a further continuously plaque pH recording for 80 min for each test.

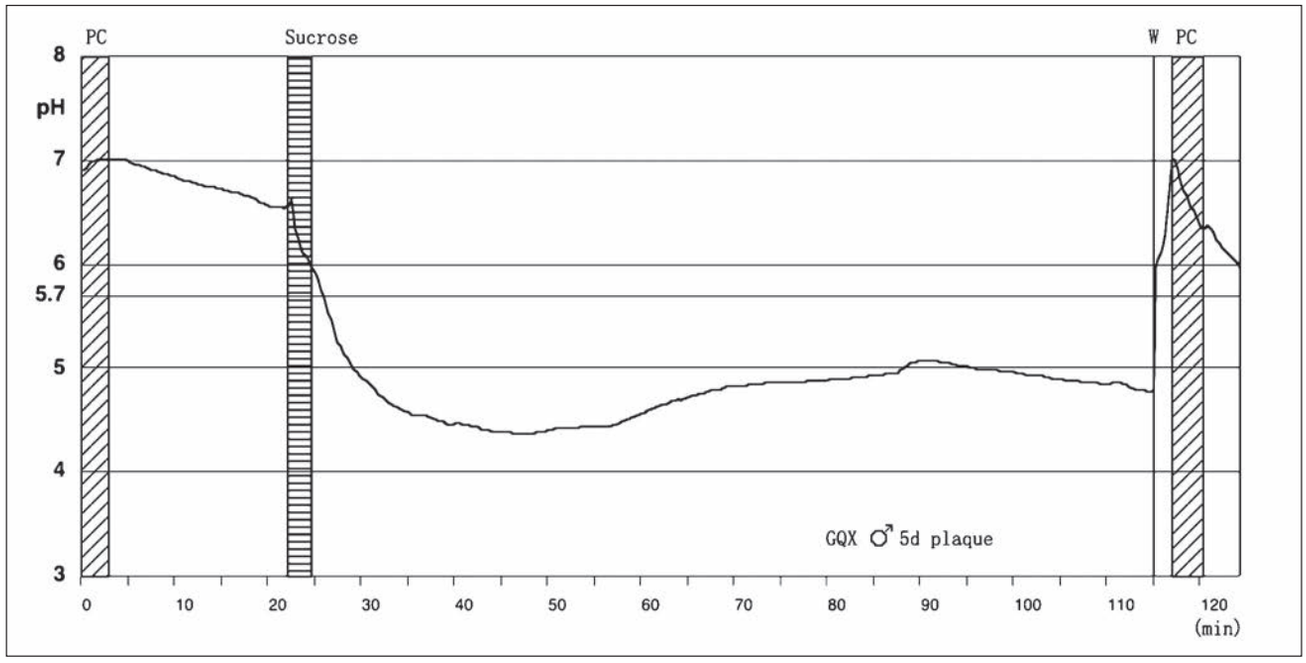

Fig 1 Telemetrically recorded interdental plaque pH profile of the subject GQX during and after sucrose rinse. W = water rinse, PC = paraffin chewing.

Table 1 Data (mean ± SD) from plaque pH response curves to sucrose rinse and 0.05% NaF rinsed at 10 min before sucrose rinse (pretreatment) and at 25 min after sucrose rinse (n = 6)

Groups	pH at 10 min after sucrose rinse	Time for plaque pH below 5.7 (min)	$AUC_{5.7}$ (arbitrary unit)
Sucrose (control)	4.36 ± 0.15	85.17 ± 10.42	77.83 ± 19.30
NaF pretreatment	4.35 ± 0.11	79.67 ± 10.28	76.42 ± 19.11
NaF post 10 min	4.35 ± 0.11	27.00 ± 7.67*	3.99 ± 3.30*
NaF post 25 min	4.37 ± 0.10	89.33 ± 13.0	57.01 ± 10.20

*$P < 0.01$ versus other groups; $AUC_{5.7}$ = area of plaque pH curve under 5.7

All NaF solutions and sucrose solutions were freshly prepared in deionized water before use. A total of 42 (6×7) telemetric curves were recorded. In all experiments, the pH values at 10 min after sucrose rinse, time for plaque pH curve below 5.7, and area of plaque pH curve under 5.7 ($AUC_{5.7}$) were calculated from the telemetric curves.

Statistical analysis

Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc). All data were presented as means ± standard deviation (SD). Two-way analysis of variance (ANOVA) was performed to compare the difference between the groups. A value of $P < 0.05$ was considered statistically significant.

Results

Sucrose rinse resulted in prolonged decrease in plaque pH

Without subsequent treatment, the plaque pH decreased to 4.36 at 10 min after the sucrose rinse and stayed below the critical 5.7 for about 85 min with $AUC_{5.7}$ at 77.83 (Fig 1, and Table 1).

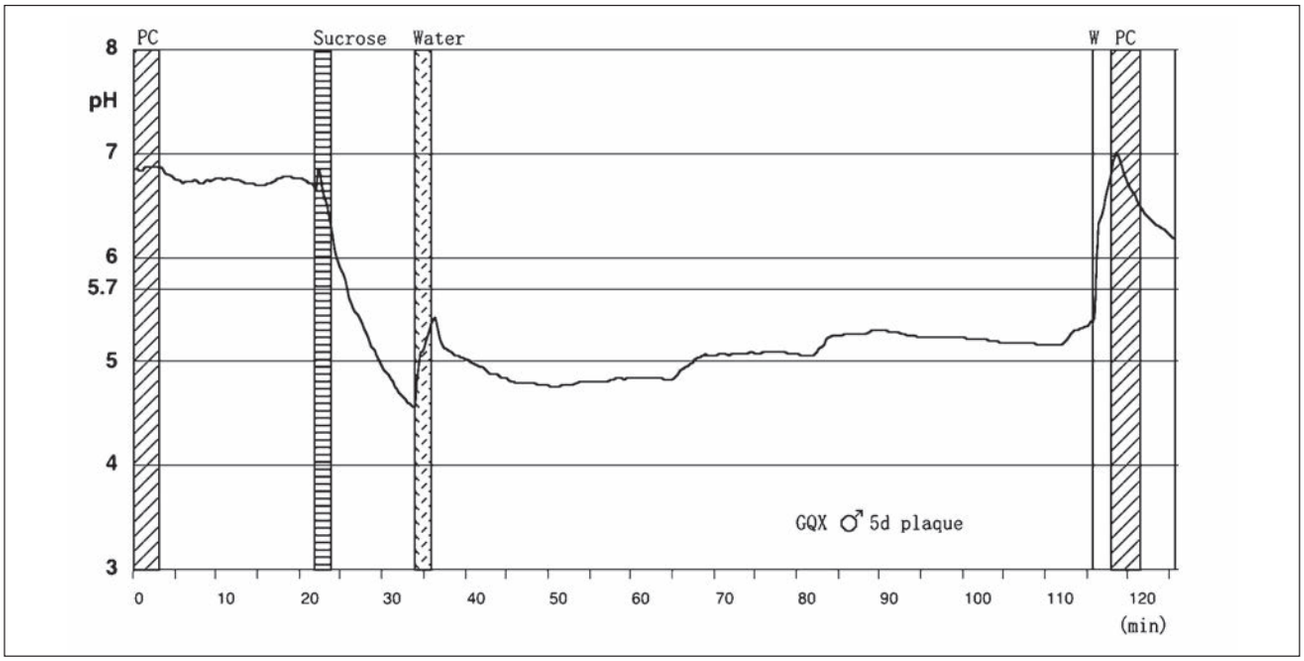

Fig 2 Telemetrically recorded interdental plaque pH profile of the same subject during and after sucrose rinse and subsequent water rinse. W = water rinse, PC = paraffin chewing.

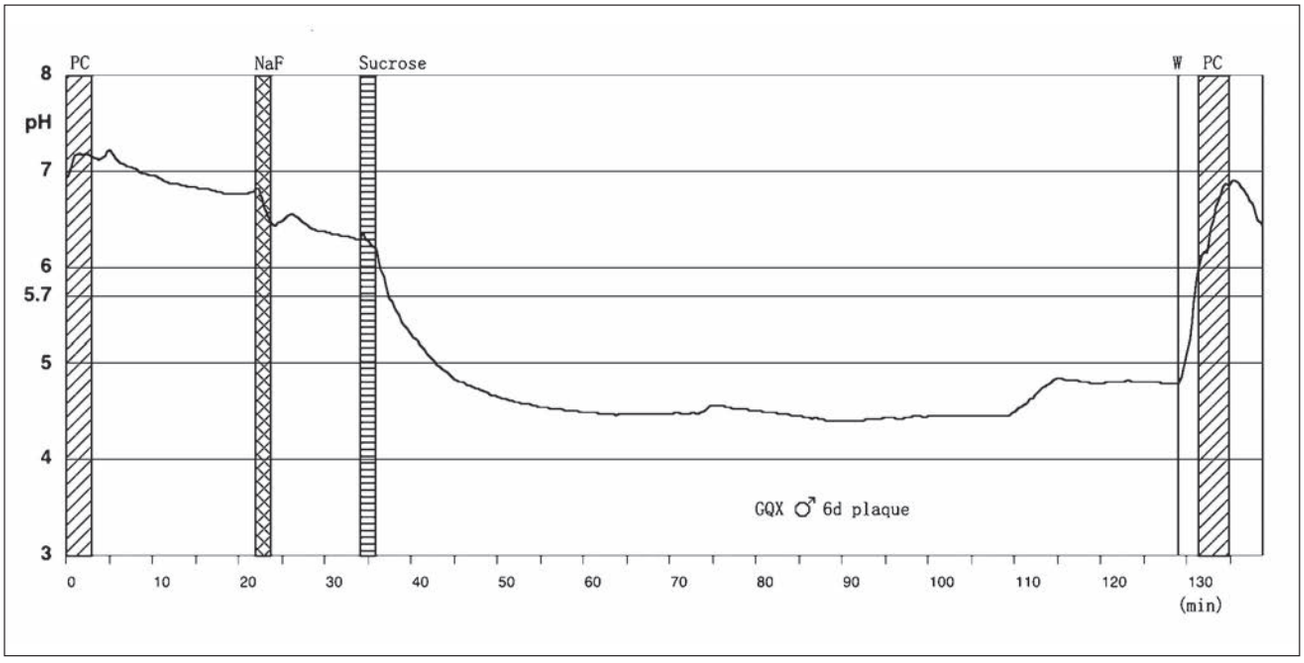

Fig 3 Telemetrically recorded interdental plaque pH profile of the same subject pretreated with 0.05% NaF solution 10 min before the sucrose rinse and during and after the sucrose rinse. W = water rinse, PC = paraffin chewing.

Table 2 Data (mean ± SD) from plaque pH response curves to sucrose rinse and subsequent water or sodium fluoride rinses at 10 min after sucrose rinse (n = 6)

Groups	pH at 10 min after sucrose rinse	Time for plaque pH below pH5.7 (min)	$AUC_{5.7}$ (arbitrary unit)
Sucrose (control)	4.36 ± 0.15	85.17 ±10.42	77.83 ± 19.30
Water	4.45 ± 0.15	77.33 ±13.50	37.47 ± 12.01
0.01% (50 ppm) F	4.33 ± 0.13	57.33 ± 23.21	26.82 ± 10.63
0.02% (100 ppm) F	4.32 ± 0.11	40.67 ± 16.94*	15.64 ± 8.27*
0.05% (250 ppm) F	4.35 ± 0.11	27.00 ±7.67*#	3.99 ± 3.30*#

*P < 0.01 versus water and sucrose groups; #P < 0.01 versus 50 ppm F; $AUC_{5.7}$ = area of plaque pH curve under 5.7

Water rinse showed little effect on sucrose-induced prolonged decrease in plaque pH

To examine the effect of a water rinse on sucrose-induced prolonged decrease in plaque pH, the water rinse was applied after the sucrose rinse. After the sucrose rinse, the plaque pH decreased to 4.45 at 10 min; after a subsequent water rinse, the plaque pH jumped close to 5.7 and decreased quickly to below 5.7 and stayed below 5.7 for about 80 min (Fig 2).

NaF pretreatment showed no effect on sucrose-induced prolonged decrease in plaque pH

A rinse with 0.05% NaF solution before a sucrose rinse showed no effect on the subsequent sucrose-induced prolonged decrease in the plaque pH, as compared the pH value at 10 min, time below critical pH 5.7, and $AUC_{5.7}$ with that of no subsequent rinse after the sucrose rinse (Fig 3 and Table 1).

NaF solution rinsed at 10 min after sucrose rinse effectively inhibited sucrose-induced prolonged decrease in plaque pH

To examine the inhibitory effect of NaF rinse on sucrose-induced decrease in plaque pH, 0.05%, 0.02%, and 0.01% NaF rinses were applied respectively 10 min after sucrose rinse. The pH of the three solutions was almost the same (pH 7.84, pH 7.68, and pH 7.83, respectively). After the sucrose rinse, the plaque pH decreased at 10 min to about 4.30 in all NaF groups, similar to that of the water and control groups, whereas after a subsequent NaF rinse, the plaque pH sharply rose to the critical 5.7 and remained around the critical 5.7 until the end of the measurement (80 min) in all NaF groups, especially remained above critical pH 5.7 for the most of measuring time in the 0.05% NaF group (Fig 4). There was a trend that the higher concentration of NaF solution was, the less time the plaque pH showed below 5.7 and the smaller the $AUC_{5.7}$ was, although there was no statistical difference between the 0.05% and 0.02% NaF groups, or between the 0.02% and 0.01% NaF groups ($P > 0.05$, Table 2), except a statistically significant difference between the 0.05% and 0.01% groups ($P < 0.01$, Table 2). However, the NaF groups, except the 0.01% NaF group, showed less time for plaque pH below 5.7 and smaller $AUC_{5.7}$, compared with that of the water and sucrose groups ($P < 0.01$, Table 2).

NaF solution rinsed at 25 min after sucrose rinse failed to inhibit sucrose-induced prolonged decrease in plaque pH

To further confirm whether the inhibitory effect of an NaF rinse on sucrose-induced decrease in plaque pH was due to the inhibition of acidogenicity of plaque by fluoride, we examined the effect of NaF solution rinsed at 25 min after the sucrose rinse; by this time, the sucrose in the plaque was almost metabolized. Rinsing with a 0.05% NaF solution at 25 min after the sucrose rinse showed no inhibitory effect on sucrose-induced decrease in plaque pH; the plaque pH curves were similar to that of the sucrose group (Fig 5) and the time for plaque pH below 5.7 and $AUC_{5.7}$ were significantly larger than that of a 0.05% NaF solution rinsed at 10 min after the sucrose rinse ($P < 0.01$, Table 1).

Discussion

In this study, we first demonstrated *in vivo* with interdental plaque pH telemetry that a single rinse of NaF solution (such as 0.05%) at 10 min after a sucrose rinse

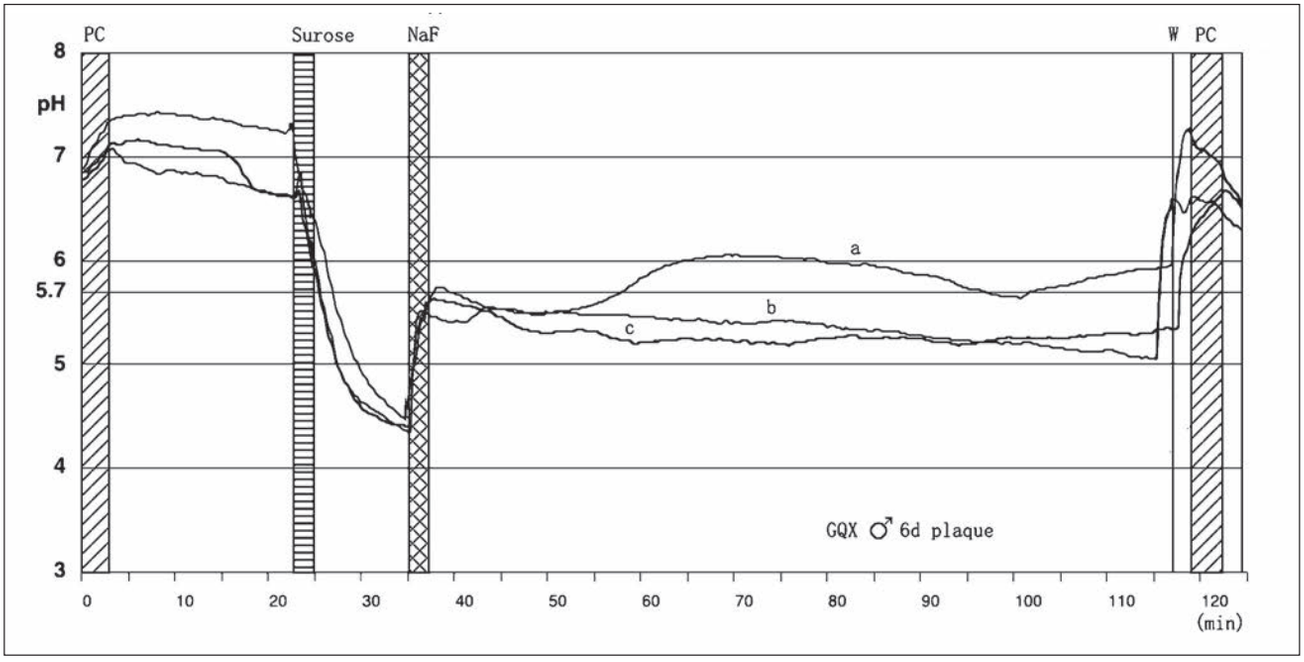

Fig 4 Telemetrically recorded interdental plaque pH profile of the same subject during and after a 0.05% (a) or 0.02% (b) or 0.01% (c) NaF rinse at 10 min after the sucrose rinse. W = water rinse, PC = paraffin chewing.

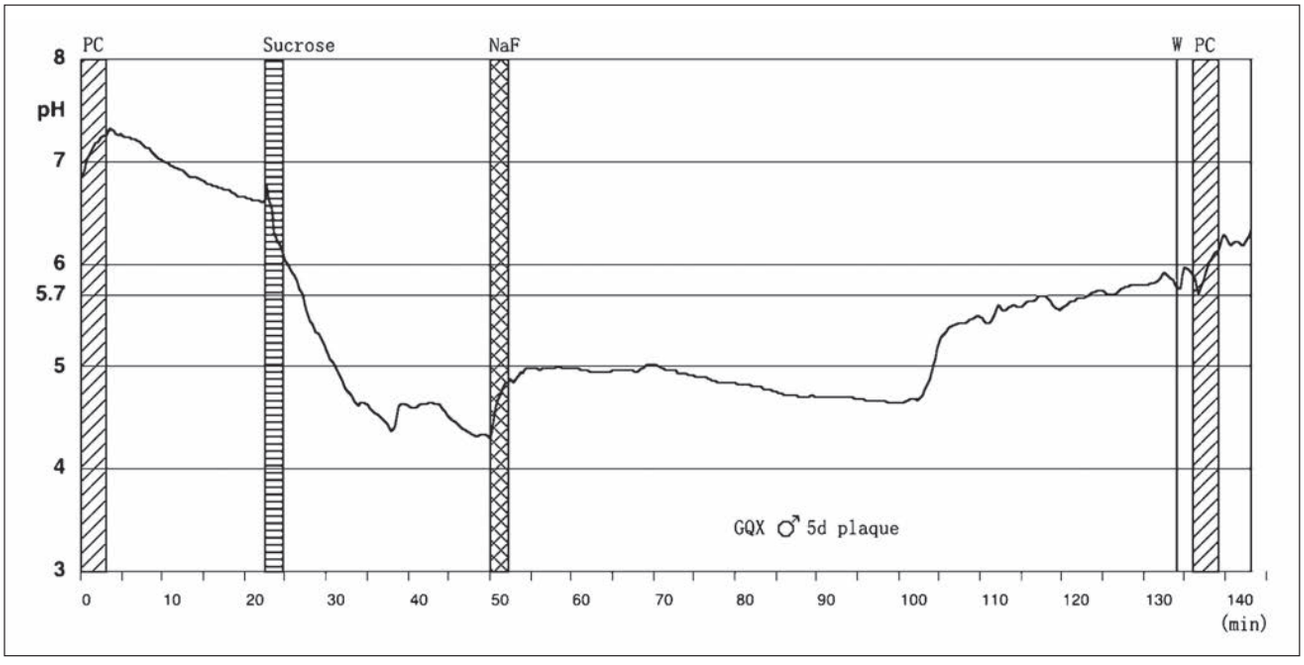

Fig 5 Telemetrically recorded interdental plaque pH profile of the same subject during and after a 0.05% NaF rinse at 25 min after the sucrose rinse. W = water rinse, PC = paraffin chewing.

effectively inhibited sucrose-induced decrease in dental plaque pH and maintained the plaque pH above the critical 5.7 for most following measuring time (80 min).

However, NaF solution rinsed before and at 25 min after the sucrose rinse showed no effect on a sucrose-induced decrease in plaque pH; water vehicle rinsing also showed little effect on the clearance of the acid in the dental plaque. These *in vivo* results indicated that rinsing daily with concentrated fluoride within 10 min after eating could effectively reduce dental plaque acidogenicity and therefore could enhance the anticaries abilities of fluoride, in addition to its main anticaries functions of inhibiting demineralization and promoting remineralization.

The mechanism underlying the inhibition of sucrose-induced decrease in plaque pH by a subsequent NaF rinse could be mainly due to the inhibitory effects of fluoride on glycolytic acid production. Previous studies *in vitro* showed that fluoride can inhibit the activities of two enzymes: enolase and the proton releasing adenosine triphosphatas, through directly binding to the two emzymes[4,5]. At low extracellular pH, fluoride is transported as HF into the bacteria cells, where it then dissociates into H^+ and F^-[22]. This process leads to an accumulation of fluoride inside the cells to inhibit glycolysis through the inhibition of the two enzymes; and simultaneously to overacidify the cytoplasm. The overacidification of the cytoplasm can further disrupt the glycolytic acid production, and the formation and catabolism of intracellular polysaccharides[4,11]. Our *in vivo* results that NaF solution rinsing at 10 min after the sucrose rinse could effectively inhibit sucrose-induced decrease in plaque pH were perfectly in accordance with, and furthermore confirmed, these results *in vitro*. In the present study, at 10 min after the sucrose rinse, the plaque pH reached a very low level – around 4.30 – due to the rapid acid production from sucrose metabolism and the slow diffusion of H^+ out of the plaque. Therefore, rinsing with an NaF solution at this moment would induce a transient plaque F^- elevation, as demonstrated previously[23], and allow more F^- to combine with H^+ forming as HF to transport into the bacterial cells, which could retard the further glycolysis and thus inhibit the acid production. However, our results of NaF solution rinsing at 25 min after the sucrose rinse failed to inhibit the sucrose-induced decrease in plaque pH. This could be due to some unfermented sugar left in plaque 20 min after the sucrose rinse[21], and also indicated that the inhibition of acidogenicity in plaque by NaF solution rinsing appeared to be dependent on the limited time period after sucrose consumption.

Although water components of fluoride solution could take away most of the sustained sucrose in the mouth and saliva to reduce any further substrate supplying the plaque, water rinsing appeared to have little effect on the acid dilution in plaque after the sucrose rinse in our study and in the previous study, even at a higher rinse frequency[18]. Therefore, our *in vivo* results indicated that fluoride solution should be rinsed within 10 min after food or carbohydrate consumption in order to have additional inhibitory effects of fluoride on acidogenicity to enhance the anticaries functions of fluoride.

However, the failure of fluoride pretreatment in inhibiting subsequent sucrose-induced decreases in plaque pH could be due to insufficient fluoride remaining in the plaque when the sucrose was rinsed. We observed that the magnitude and duration of inhibitory effects of fluoride rinsed after the sucrose on plaque acidogenicity depended on its concentration, i.e. the lowest concentration of fluoride (0.01%) showed only a marginal inhibitory effect on a sucrose-induced decrease in plaque pH, whereas the highest concentration of fluoride (0.05%) showed the strongest inhibitory effect, suggesting that sufficient fluoride remaining or diffusing in the plaque when the sucrose was fermented was critical for fluoride to inhibit plaque acidogenicity. The reason why the highest concentration of fluoride pretreated before the sucrose rinse did not inhibit subsequent sucrose rinsing induced decrease in plaque pH could be due to the washout by the subsequent sucrose rinse and also the possible diluting by the saliva, leading to insufficient fluoride remaining in the plaque, and finally failure in inhibiting the subsequent plaque acidogenicity when the sucrose was rinsed.

A daily use concentration of fluoride (0.05%) rinsing after food or carbohydrate consumption was suggested for caries prevention. In the present study, we observed that 0.05% (250 ppm) was the most effective concentration for NaF solutions rinsed after the sucrose rinse, compared with the 0.02% (100 ppm) and 0.01% (50 ppm) groups. It was not excluded that fluoride solutions with higher concentrations could have higher inhibitory effects on plaque acidogenicity than the 0.05% solution did. However, 0.05% or 250 ppm is suggested to be the daily used concentration of fluoride recommended to prevent caries in high-risk patients[24]. Considering that the lower the fluoride concentration, the less the fluoride penetrates into the plaque[25], and the trend of the inhibitory effects on plaque acidogenicity was fluoride concentration dependent in the present study, 0.05% fluoride solution rinsing after food was suggested to exert the dual roles (mineral effects and microbial actions) of fluoride for caries prevention. Although a concentration (0.02% or 100 ppm) less than

0.05% for fluoride was also suggested to sufficiently reduce the virulence factors based on the results from the single species biofilm model[9], the penetration of fluoride into dental plaque could be more limited than in the biofilm model, because the structure and permeability and composition of microbial community of the biofilm model was different from our interproximal dental plaque. Therefore, the fluoride concentration of 0.05% (250 ppm) for rinsing after eating could sufficiently decrease the demineralisation potential and be more beneficial for caries prevention.

The interproximal area is more susceptible to caries than the buccal or lingual smooth surfaces, because the plaque in this site is more difficult to remove, and the diffusion in or out of the plaque is slower, and saliva contact is less. This may affect the topical effectiveness of an antimicrobial mouth rinse. In the present study, the pH change was recorded continuously under undisturbed interproximal plaque with an indwelling electrode system. Therefore, the method we used may be advantageous in specifically and sensitively evaluating the acidogenic property of plaque and the inhibitory effect of fluoride on plaque acidogenicity.

In conclusion, rinsing with 0.05% fluoride after a sucrose rinse could significantly reduce the acidogenicity of the interproximal dental plaque, largely decrease the demineralization potential and therefore enhance the anticaries functions of fluoride.

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