Lower Fluoride-, Calcium- and Phosphate-containing Rinse More Effectively Enhances Fluoride Uptake in Plaque than NaF Rinse

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Objective: *To evaluate the effects of a remineralisation solution (RS) containing lower fluoride (F), calcium (Ca) and phosphate (P) in comparison with conventional NaF rinse on F and Ca uptake by dental plaque, and on acid resistance and remineralisation of enamel.*
Methods: *Eight subjects participated in this randomised, double-blind and cross-over* in vivo *study. Subjects wore removable appliances mounted with a pair of enamel blocks and applied the RS rinse and conventional NaF rinse three times per day for 1 week, repectively. The RS rinse, pH 6.5, contained 0.53 mmol/L F of NaF, 15 mmol/L* $CaCl_2$*, and 9 mmol/L* NaH_2PO_4*. The NaF rinse contained 12 mmol/L F. Dental plaque was accumulated for 48 h and collected after fasting overnight after the last use of the rinse. After acid extraction, F and Ca in the plaque were measured by an ion-selective electrode and atomic absorption spectroscopy, respectively. Acid resistance of the enamel was evaluated by microradiography after the enamel blocks were artificially demineralised by acid gel* in vitro. *The remineralisation of the pre-softened enamel was determined using a microhardness test.*
Results: *Both rinses significantly enhanced F and Ca uptake in whole plaque. RS rinse produced a similar F deposition in plaque to the NaF rinse, although the formulation of RS rinse contained 22.6 times less F than the NaF rinse did. Moreover, RS rinse more effectively enhanced acid resistance and remineralisation of enamel than the NaF rinse did.*
Conclusion: *RS rinse can provide greater cariostatic effects with a lower F dosage than the conventional NaF rinse, with less worry about excessive systemic F intake in children.*
Key words: *fluoride, dental plaque, mouth rinse, acid resistance, remineralisation*

Demineralisation and remineralisation are two dynamic processes of dental caries. Caries occurs when demineralisation is not fully reversed by remineralisation. Fluoride (F) is an effective anti-caries agent because it can inhibit demineralisation and promote remineralisation. Previous *in vitro* studies showed that a small increase in the fluid concentration of F can have a profound anti-caries effect[1,2]. Clinical studies also demonstrated an inverse relationship between F in dental plaque and the prevalence of caries levels[3-5]. It was also known that F deposition in dental plaque is an important F reservoir, which may provide a significant source of F for oral fluids when the pH falls during eating and drinking[6,7]. It was suggested, therefore, to increase the uptake and retention of F in dental plaque to increase the cariostatic effectiveness of F.

One obvious way to increase F concentration in dental plaque is to increase F content in dental products. This approach, however, has the disadvantage of increasing the potential risk of increasing the severity and incidence of dental fluorosis because of the

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excessive systemic F intake in young children. It was observed that the retention of F in dental plaque is mainly dependent on plaque calcium (Ca) concentration[8-11]. However, there is controversy over whether a Ca-containing rinse could enhance F uptake in dental plaque[12,13].

Previous studies have shown that a Ca- and phosphate (P)-containing rinse with a lower concentration of F, called a remineralisation solution (RS) rinse, can inhibit demineralisation and promote remineralisation *in vitro*[14] and also has a significant effect on anti-radiation caries *in vivo*[15]. However, the mechanism underlying the anti-caries effects of RS rinse remains unknown. The purpose of this study was to evaluate the effects of RS rinse in comparison with conventional NaF rinse on F and Ca uptake by dental plaque and on acid resistance and remineralisation of enamel after being applied by subjects.

Materials and Methods

Mouth rinse composition

Two mouth rinses were prepared in distilled water. The NaF rinse consisted of 12 mmol/L F, pH 6.7, according to a current over-the-counter formulation, whereas the RS rinse, pH 6.5, contained 0.53 mmol/L F of NaF, 15 mmol/L $CaCl_2$ and 9 mmol/L NaH_2PO_4. The RS rinse was prepared by mixing individual solutions of the above components in a special sequence; no precipitation was observed during the period of the experiment.

Subjects

Eight healthy adult subjects (six females and two males) ranging in age from 20 to 45 years were recruited from the staff of Peking University School of Stomatology. Ethical approval for this study was obtained from the Ethical Committee of Peking University Health Science Center, and all subjects provided informed consent. None of the subjects had unfilled cavities, periodontal disease or other oral diseases. At the beginning of the study, all of the subjects received professional tooth cleaning to remove all accessible calculus and plaque. The subjects were instructed not to use F-containing products and not to drink tea during the experiment period.

The study was a double-blind, randomised, and cross-over design. Neither the investigators nor the subjects were aware of the identities of the rinses. The study was conducted over a 4-week period; 1 week each was assigned for each period of baseline, NaF rinse, wash out, and RS rinse. The subjects served as their own controls and were asked to randomly take one of the two rinses to wash mouth with 10 ml for 1 min three times per day (in the morning, after lunch and before sleep) for 1 week. Wash out was defined as the subjects using no rinse for 1 week before taking the second rinse.

Collection and analysis of dental plaque samples

Dental plaques were collected before the use of rinse (baseline) and after the use of the rinses and wash out. The subjects were instructed to refrain from oral hygiene to accumulate plaque for 48 h and to fast overnight until plaque collection the next morning. Pooled plaque samples were taken from accessible molar and premolar tooth surfaces with a dental scalar and placed inside the preweighed microcentrifuge tubes to reweigh for wet weight of the plaques with an electronic balance (accuracy of ± 0.00001 g). Care was taken to avoid any food debris or blood.

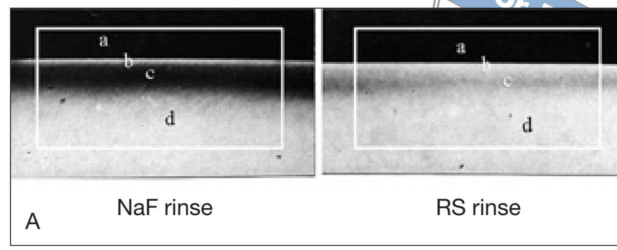
The plaque samples were dried sufficiently over P_2O_5 and extracted in 600 µl of 0.5 mol/L $HClO_4$ at room temperature for 12 h. F was estimated with an ion-selective electrode (Cole–Parmer A-27502-19, USA) after neutralisation of 100 µl samples with 10 µl of 5 mol/L NaOH and addition of 100 µl low-level total ionic strength adjustment buffer containing a background level of 2.04 µmol/L F[16]. Acid-extractable Ca was determined by atomic absorption spectroscopy (Avanta PM, GBC, Australia). The Ca and F values were expressed on a wet-weight basis.

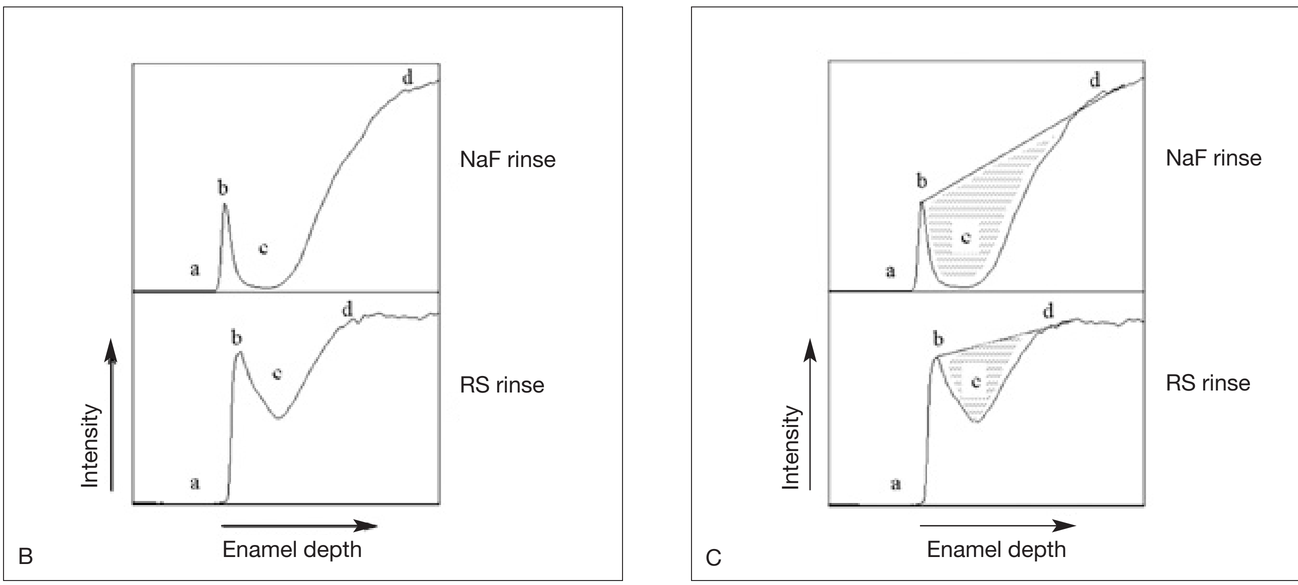
Acid resistance and remineralisation

Preparation of tooth specimens

The experiment was carried out using the intraoral caries test. Two enamel blocks with intact surfaces of 2 mm × 3 mm were cut from the lingual and buccal surfaces of the crown of a premolar extracted for orthodontic reasons. The two enamel blocks were used as a matched pair and a total of 16 pairs of blocks were prepared and randomly and equally assigned for the acid resistance and remineralisation experiments. For the remineralisation experiment, the enamel blocks were first placed in an acid gel containing 3.0% hydroxyethyl cellulose and 0.1 mol/L lactic acid, pH 4.0, for 48 h at 40 °C to be softened (forming artificial lesions as described previously)[17]. The microhardness of the enamel blocks was measured by a microhardness tester (HX-1000, Shanghai, China) before use of the rinse.

Fig 1 RS rinse more effectively enhanced acid resistance of enamel than NaF rinse. (A) Microradiographs of enamel blocks demineralised in the acid gel after use of the NaF or RS rinse for 1 week *in vivo*; a–d: background, surface layer of the lesion, subsurface layer of the lesion and sound enamel, respectively. (B) Plots of intensity versus depth of enamel; a–d as in (A). (C) The shadowed area was calculated to reflect the mineral lost in the lesion. The larger the shadowed area was, the less mineral content there was in the lesion.





Intraoral appliance
Removable mandibular arch acrylic appliances were constructed for the right premolar and first molar. For each rinse test, one intact enamel block (for acid resistance) and one presoftened enamel block (for remineralisation) were mounted on the holes prepared in the lingual surfaces of the appliance. The appliance was worn by the subjects day and night during the period of rinse. At the end of 1 week of using the rinse, the two blocks were removed for evaluation of acid resistance or remineralisation.

Quantitative assessment of mineral contents
For the acid resistance experiment, after use of each rinse the enamel blocks were placed in the acid gel for 48 h at 40 °C to make artificial subsurface lesions. The enamel blocks were further prepared for microradiography as described in detail in a previous study[17]. The radiographs were scanned into a computer and analysed with the image analysis software Image J Version 1.37v (NIH, for free public use). For each radiograph, the same rectangular region of interest was duplicated to cover the background (a), the surface layer of lesion (b), the subsurface layer of lesion (c) and the sound enamel (d), as shown in Fig 1A, and a plot of intensity versus enamel depth was obtained (Fig 1B). The plot also showed four segments (a to d) corresponding to the background, the surface layer of lesion, the subsurface layer of lesion and the sound enamel (Fig 1B). The subsurface lesion area corresponded to the shadowed area under the line from the peak of the surface to the sound enamel (Fig 1C). The shadowed area was calculated by the software automatically to reflect the mineral lost in the lesion. The larger the shadowed area was, the less mineral content was in the lesion. For the remineralisation experiment, the microhardness value of the presoftened enamel blocks was remeasured after use of the rinse. The remineralisation of enamel blocks with artificial lesions could be reflected by the changes in microhardness values.

Statistical analysis

Statistical analysis was performed with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA). All data were presented as means plus/minus standard deviation. Independent group one-way analysis of variance was performed for

Table 1 Concentrations of F and Ca in whole plaque (ng/mg wet weight, n = 8)

	Baseline	NaF rinse	Wash out	RS rinse
F	8.36 ± 1.80	17.31 ± 2.10*	8.97 ± 6.50	15.63 ± 3.11*
Ca	196.16 ± 76.18	343.11 ± 128.34*	94.50 ± 49.93	569.19 ± 176.07*, #

**P < 0.01 versus baseline and wash out; #P < 0.01 versus NaF rinse.*

comparing the concentrations of F and Ca. An independent t-test was performed for the shadowed area and microhardness between rinses. A paired *t* test was performed for microhardness before and after use of the rinse. A value of $P < 0.05$ was regarded as statistically significant.

Results

Enhancement of F and Ca uptake in whole plaque after use of two rinses

As shown in Table 1, the concentrations of both F and Ca in whole plaque were significantly increased after the subjects applied the two rinses for 1 week, compared with the baseline (P < 0.01). The concentrations of F and Ca were increased 2.0 and 1.7 times respectively after use of NaF rinse, and by 1.8 and 2.9 times respectively after use of the RS rinse. There was no statistically significant difference in F concentration in plaque after use of the two rinses (P > 0.05), although the formulation of the RS rinse contained 22.6 times less F than the NaF rinse. Similarly, the use of NaF rinse increased by 1.7 times the Ca in the plaque, eventhough its formulation did not contain Ca. However, the concentration of Ca in plaque after use of the NaF rinse was still much lower than that after use of the RS rinse (P < 0.01; Table 1). Both concentrations of F and Ca in the plaque were reduced to the baseline after 1 week of not using either rinse (wash out) (P > 0.05).

RS rinse more effectively enhanced acid resistance of enamel than NaF rinse

To test the anti-caries effects of the rinses, the resistance to acid infusion by the enamel blocks was observed. The model of an artificial enamel subsurface lesion was used for acid resistance. After treating with each rinse for 1 week in the oral appliance, the enamel blocks were demineralised in acid gel to form artificial subsurface lesions. As shown in Fig 1A, the enamel blocks showed less severe subsurface lesions after use of the RS rinse than after use of the NaF rinse. The lesion area of the enamel block after use of the RS rinse was 3346 ± 1434, which was significantly smaller than the lesion area of 7060 ± 2531 after use of the NaF rinse (P < 0.05).

RS rinse more effectively enhanced remineralisation of enamel than NaF rinse

The microhardness is an important parameter to reflect the mineral content or remineralisation of enamel. Enamel blocks were presoftened before use of the rinses in the oral cavities of subjects who applied each rinse for 1 week and then measured the microhardness. As shown in Table 2, the microhardness of the enamel was significantly increased after use of either rinse. Moreover, the microhardness of the enamel after use of the RS rinse was greater than that after use of the NaF rinse (P < 0.01).

Discussion

In the present study, it was found that both the RS rinse and the NaF rinse could significantly enhance the concentrations of F and Ca in whole plaque after being applied by the subjects for 1 week. Higher concentrations of F and Ca in dental plaque are important for enamel demineralisation protection. Moreover, the RS rinse was more effective in increasing Ca in the plaque than the NaF rinse (Table 1) and it correspondingly also more effectively enhanced acid resistance and remineralisation of the enamel than the NaF rinse after being applied by the subjects (Fig 1 and Table 2). The results suggest that the RS rinse could be a better mouth rinse for caries prevention than the conventional NaF rinse. Given that the RS rinse contained a much lower concentration of F than the NaF rinse (22.6 times lower) or other Ca/F-containing rinses[18,19], there would be less worry regarding increasing the severity and incidence of dental fluorosis in children using this rinse for caries prevention.

Ca greatly increased F deposition in dental plaque. Although the RS rinse contained 22.6 times lower F than the NaF rinse, it surprisingly had a similar ability to allow F to deposit in plaque as the NaF rinse did (P > 0.05; Table 1). Considering that the concentration of Ca in plaque was 2.9 times higher than the baseline after

Table 2 Microhardness values (HV) of enamel before and after use of NaF or RS rinse (n = 8)

Group	Before	After
NaF rinse	46.67 ± 11.08	71.69 ± 31.04*
RS rinse	48.06 ± 8.85	95.32 ± 45.85*,#

**P* < 0.01 versus before; #*P* < 0.01 versus NaF rinse.*

the subjects used the RS rinse and was also higher than that in plaque of the subjects applying the NaF rinse (Table 1; $P < 0.01$), this suggests that F uptake by plaque was boosted by Ca in the plaque. The results of Ca enhancement of F deposition in plaque supports previous suggestions by other researchers that F deposition in plaque is dependent more on the amount of Ca in the plaque than on the concentration of F in the rinse[8,11,20]. Although P was not examined in the present study, it was possible that P would be increased in the dental plaque after use of the P-containing RS rinse. It is not ruled out that P may also to some extent help increase F and Ca deposition in plaque through forming P-containing CaF_2 or F-containing calcium phosphate salts.

However, the precise mechanism underlying Ca enhancement of F deposition in plaque is not fully understood. It has been established that the F deposition in plaque is limited by the low level of Ca available in oral fluids after an NaF rinse[21]; the neutral pH of the NaF rinse also limits the amount of Ca supplied by enamel dissolution. In addition, the fraction of total plaque Ca involved in the uptake and retention of F is also relatively small[21]. This could be the reason why F uptake by plaque is not proportional to F supplied in the rinse. In fact, it was found that consumption of higher F dentifrice does not proportionally produce higher F in plaque. It is believed that, during the use of the higher F dentifrice, the salivary F concentration greatly exceeds the salivary Ca concentration and causes the formation of large amounts of CaF_2, which accumulates on the surface of the plaque and restricts the penetration and uptake of the ions into the interior of plaque, which are instead swallowed or expectorated[11,13]. In the present study, the RS rinse was formulated with Ca and much lower F so that the formation of relatively insoluble CaF_2 would be prevented before the F and Ca ions formed as CaF^+ and penetrated into the plaque, and this is easy for the reaction of the ions in the plaque[22,23]. This may somehow explain why the RS rinse produced comparable F and higher Ca in the plaque compared with the NaF rinse. The results for the RS rinse are consistent with the results of a previous study, in which a rinse consisting of 3 mmol/L F and 200 mmol/L $CaCl_2$ produced the same protection against demineralisation as did the 13.2 mmol/L F NaF rinse[18]. However, compared with this study, F and Ca was further decreased in the RS rinse but with addition of P and it showed even better effects than the NaF rinse on acid resistance and remineralisation of enamel.

F also enhanced Ca uptake in plaque. Although the NaF rinse did not contain Ca, the concentration of Ca in plaque was increased 1.7 times after the subjects used the NaF rinse (Table 1). This phenomenon was also observed in previous studies[12,13,24]. It is believed that the phenomenon was due to the formation of positively charged calcium fluoride ions (CaF^+) in saliva that would be attracted to negatively charged species within plaque and on the enamel surface. It is believed that F can promote the migration of Ca from saliva to plaque. For instance, it was found that the Ca binding capacity by *Streptococcus mutans* was doubled when F was added[9]. Therefore, with the deposition of F in plaque increased 2.0 times, the deposition of Ca was also proportionally increased 1.7 times in plaque (Table 1) after use of the NaF rinse. This may also explain the reason why the NaF rinse enhanced remineralisation of the enamel (Table 2).

Higher plaque levels of Ca, F, and P ions could result in a higher degree of saturation in plaque fluid with respect to enamel minerals, thereby lowering the risk of enamel demineralisation and facilitating remineralisation. Although P was not examined in plaque, it was reasonable to suppose that the P concentration would increase after use of the RS rinse. The RS rinse provided Ca, F, and P ions capable of diffusing into plaque and the surface of enamel, therefore promoting remineralisation of enamel (Fig 1 and Table 2) to provide a high level of caries protection at a lower F dosage.

In conclusion, this study shows that the RS rinse containing much lower F, Ca, and P produced greater remineralisation than the conventional 12 mmol/L F NaF rinse. The reduced F content in the RS rinse could also be advantageous in minimising excessive systemic F intake in children. The results suggest that the RS rinse could provide greater cariostatic effects with a lower F dosage than a conventional NaF rinse.

References

1. Margolis HC. An assessment of recent advances in the study of the chemistry and biochemistry of dental plaque fluid. J Dent Res 1990;69:1337–1342.
2. Ten Cate JM. *In vitro* studies on the effects of fluoride on de- and remineralization. J Dent Res 1990;69:614–619; discussion 634–616.
3. Ashley FP. Calcium and phosphorus concentrations of dental plaque related to dental caries in 11- to 14-year-old male subjects. Caries Res 1975;9:351–362.
4. Schamschula RG, Bunzel M, Agus HM, Adkins BL, Barmes DE, Charlton G. Plaque minerals and caries experience: associations and interrelationships. J Dent Res 1978;57:427–432.
5. Wilson RF, Ashley FP. The relationship between the biochemical composition of dental plaque from both approximal and free smooth surfaces of teeth and subsequent 3-year caries increment in adolescents. Arch Oral Biol 1990;35:933–937.
6. Ogaard B, Rolla G, Helgeland K. Uptake and retention of alkali-soluble and alkali-insoluble fluoride in sound enamel *in vivo* after mouthrinses with 0.05% or 0.2% NaF. Caries Res 1983;17:520–524.
7. Rolla G, Saxegaard E. Critical evaluation of the composition and use of topical fluorides, with emphasis on the role of calcium fluoride in caries inhibition. J Dent Res 1990;69:780–785; discussion 820–823.
8. Rose RK, Hogg SD, Shellis RP. A quantitative study of calcium binding by isolated streptococcal cell walls and lipoteichoic acid: comparison with whole cells. J Dent Res 1994;73:1742–1747.
9. Rose RK, Shellis RP, Lee AR. The role of cation bridging in microbial fluoride binding. Caries Res 1996;30:458–464.
10. Vogel GL, Zhang Z, Chow LC, Schumacher GE. Effect of a water rinse on ‘labile’ fluoride and other ions in plaque and saliva before and after conventional and experimental fluoride rinses. Caries Res 2001;35:116–124.
11. Whitford GM, Wasdin JL, Schafer TE, Adair SM. Plaque fluoride concentrations are dependent on plaque calcium concentrations. Caries Res 2002;36:256–265.
12. Pessan JP, Sicca CM, de Souza TS, da Silva SM, Whitford GM, Buzalaf MA. Fluoride concentrations in dental plaque and saliva after the use of a fluoride dentifrice preceded by a calcium lactate rinse. Eur J Oral Sci 2006;114:489–493.
13. Whitford GM, Buzalaf MA, Bijella MF, Waller JL. Plaque fluoride concentrations in a community without water fluoridation: effects of calcium and use of a fluoride or placebo dentifrice. Caries Res 2005;39:100–107.
14. Chen XQ, Wang Q. Microhardness measurement and electron analysis of the effects of NaF and remineralizing solution on enamel early lesions. Beijing Yi Ke Da Xue Xue Bao 1995;27:285–287 (in Chinese).
15. Zhang J. Prevention of dental caries in irradiated cancer patients with a remineralization solution. Thesis of Beijing Medical University School of Stomatology 1995 (in Chinese).
16. Pearce EI, Nelson DG. *In vivo* comparison of caries inhibition by a plaque mineral enriching mouthrinse and a fluoride dentifrice. Caries Res 1988;22:362–370.
17. Wang XL, Gan YH, Ge Y, Cheng CY, Chen XC, Wang WH, Zhang BX. Remineralization of enamel subsurface lesions by xylitol chewing gum containing calcium hydrogen phosphate and fouoran. Chin J Dent Res 2007;10:38–42.
18. Takagi S, Liao H, Chow LC. Effect of a low-fluoride-content, two-component rinse on fluoride uptake and on de- and remineralization of enamel lesions: an *in vitro* study. Caries Res 2001;35:223–228.
19. Vogel GL, Mao Y, Carey CM, Chow LC. Increased overnight fluoride concentrations in saliva, plaque, and plaque fluid after a novel two-solution rinse. J Dent Res 1997;76:761–767.
20. Vogel GL, Mao Y, Carey CM, Chow LC, Takagi S. *In vivo* fluoride concentrations measured for two hours after a NaF or a novel two-solution rinse. J Dent Res 1992;71:448–452.
21. Margolis HC, Moreno EC. Composition and cariogenic potential of dental plaque fluid. Crit Rev Oral Biol Med 1994;5:1–25.
22. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 2003;82:206–211.
23. Vogel GL, Zhang Z, Chow LC, Carey CM, Schumacher GE, Banting DW. Effect *in vitro* acidification on plaque fluid composition with and without a NaF or a controlled-release fluoride rinse. J Dent Res 2000;79:983–990.
24. Vogel GL, Schumacher GE, Chow LC, Takagi S, Carey CM. Ca pre-rinse greatly increases plaque and plaque fluid F. J Dent Res 2008;87:466–469.