The effects of toothpaste amounts and post-brushing rinsing methods on salivary fluoride retention

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Abstract

Purpose – The purpose of this paper is to investigate the salivary fluoride retention as fluoride concentration, amount of soluble fluoride, half-life ($t_{1/2}$) and salivary flow rate of different amounts of toothpaste and rinsing procedures.

Design/methodology/approach – A randomized crossover study of 21 healthy volunteers was designed to compare pharmacokinetic parameters of 1 g (B1) and 0.3 g (B0.3) of toothpaste without rinsing and brushing with 1 g of toothpaste with expectoration followed by water rinsing (B1R). Unstimulated saliva was collected before brushing as a baseline and at 0, 5, 10, 30, 60 and 90 min after the completion of the tooth brushing procedure.

Findings – The salivary fluoride concentration and amount of soluble fluoride of the B1 group were significantly higher than the B0.3 and B1R groups. The B1 and B1R groups prolonged the remineralizing level up to 60 min while the B0.3 group retained their remineralizing levels for 30 min. The initial $t_{1/2}$ (rapid phase) of B1 and B1R groups were significantly longer than the B0.3 group. The late $t_{1/2}$ (slow phase) of the B0.3 group was significantly longer than the B1 group. This is called the two-compartment open pharmacokinetics model. There was no statistical difference of salivary flow rates between all groups.

Originality/value – Non-rinsing and the amount of fluoride toothpaste play an important role in raising salivary fluoride levels and prolonging the remineralizing level of the oral cavity.

Keywords Dry brushing, Fluoride retention, Fluoride toothpaste, Pharmacokinetic model, Dental hygiene, Mouth rinsing

Paper type Research paper

Introduction

Dental caries is a disease caused by bacteria that produces and releases acid into the biofilm and saliva and dissolves the crystal structure of enamel (hydroxyapatite). Fluoride (F) is widely used to reduce the prevalence of dental caries[1] by inhibiting demineralization and promoting remineralization[2]. In acidic environments, hydroxyapatite can dissolve the optimal pH which releases Ca^{2+} and PO_4^{3-} into the environment. If there is F present, it can penetrate into the tooth enamel and absorb hydroxyapatite and protect it from dissolution[2]. Also, F can protect dental caries by remineralizing Ca^{2+}, PO_4^{3-} and F^- into fluorapatite, which makes the enamel more resistant to further acid exposure[2]. Effective low F concentrations that can inhibit demineralization and enhance remineralization are at 0.02 ppm and 0.04 ppm concentration levels, respectively[3]. Thus, F has been added in many

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home-use oral care products such as toothpastes and mouthwashes[4]. F toothpaste is the recommended strategy to maintain good oral health in children and adolescents[5] and reduce dental caries and have been used for many decades around the world[6]. Also, the effectiveness of low F concentrations in saliva was found after the use of F toothpaste[3]. The factors that increased F retention in order to obtain the best results was F concentration > amount of toothpaste > rinsing procedure[7]. The systematic review of F toothpaste in different concentrations indicated that the F concentration of 1,000 ppm or higher could prevent dental caries in children and adults[8]. The use of 1,000 ppm of F toothpaste in children under six years of age must be well-instructed and monitored because children tend to swallow toothpaste and dental fluorosis might occur[9]. In Thailand, there are two concentrations of F in toothpaste, e.g., 500 ppm for children and 1,000 ppm for adults. The recommendations of the amount of 1,000 ppm F toothpaste are 1 g for adults[10] and 0.3 g for young children[11].

F retention in the oral cavity is influenced by many factors such as saliva clearance, F concentration of toothpaste, amount of toothpaste and water rinsing[7, 12, 13]. Recently, using 1 g of F toothpaste or approximately the full length of the brush head, followed by expectoration without water rinsing[4] was recommended in order to prolong F levels in the oral cavity. This technique is called dry brushing. However, some people feel comfortable and clean when rinsing after brushing[14]. A further study recommended rinsing with a small amount of water after brushing which had the similar F retention as dry brushing[15]. This method is an alternative for people unable to follow the "spit, don't rinse" method. Moreover, most of the toothpaste used is spat out, and as a result, the actual amount needed to be used may be less than the recommended amount. However, it is unknown if the F retention from dry brushing with < 1 g of toothpaste would result in the retention of F levels required for tooth remineralization. It would be economical and might reduce the risk of excessive F ingestion, especially in children.

The aim of this study was to investigate salivary F retention demonstrated by pharmacokinetic parameters: F concentration and F half-life in saliva after brushing with 1 or 0.3 g of F toothpaste without rinsing or 1 g F toothpaste with rinsing. The salivary flow rate using the different brushing and rinsing procedures was also studied.

Materials and methods

Ethics and clinical trial consideration

The study protocols were approved by The Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (No. 044/2017) and The Thai Clinical Trials Registry, Thailand (TCTR20180619006). Informed consent was obtained from all participants.

Participants

The study was conducted in the laboratory of the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. The 21 volunteers were healthy adults (10 males and 11 females) aged 18–23 years old and living in Bangkok, Thailand. The inclusion criteria were good general and dental health; 28 permanent teeth fully erupted not including third molars (to avoid the effect of fluoride reservoir between teeth); not wearing fixed orthodontic appliance or a removable denture; an unstimulated whole salivary flow of 0.3–1.1 ml/min; and daily brushing with F toothpaste and no history of using dry brushing technique. The exclusion criteria were unable to participate for the duration of the full experiment period and could not comply with the study protocol. The participants were involved in the experiment for 16 days. Power analysis determined that 21 volunteers were required to demonstrate a 5 percent difference in mean salivary F concentrations among the three groups with 80 percent power ($\alpha = 0.05, \beta = 0.20$)[16]. The volunteers were carefully selected in order to comply with the instructions given.

Oral fluoride clearance study

The study was blinded to the examiners and used a crossover design experiment (Figure 1). Participants were allocated into different sequences of brushing methods by simple randomization. Investigators trained the modified Bass technique to all participants by teaching one-on-one, supplying written instructions and asking each participant to perform in front of investigators until they achieved the required skill. One week before the experiment, participants brushed their teeth with 1 g (0.8 ml in 2 ml syringe) of 1,000 ppm F toothpaste (Darlie Thailand® Zesty fresh mint toothpaste, Siam Regal Co. Ltd, Bangkok, Thailand), using the modified Bass technique for 2 min with 10 ml in a measuring cup including post-brushing rinsing for 10 s. Participants were instructed to avoid F-rich foods and beverages during the experiment, not to use any other F products apart from the 1,000 ppm F toothpaste and not to eat or drink at least half an hour after tooth brushing. They were assigned to brush their teeth with three methods sequentially according to their randomly allocated experimental session. The three brushing methods were (B1) brushing with 1 g of toothpaste without rinsing (only expectoration), (B0.3) brushing with determined minimal amount of toothpaste (0.3 g) without rinsing (only expectoration) and (B1R) brushing with 1 g of toothpaste followed by 5 ml water rinsing as slurry within 10 s. During each three-day washout period, the participants brushed with 1 g of F toothpaste and 10 ml of water rinsing in the pre-experiment phase. The 1,000 ppm F toothpaste used in a preparation and experiment session was the same brand and manufacturing lot number throughout the study (Darlie Thailand® Zesty fresh mint toothpaste, Siam Regal Co. Ltd, Bangkok, Thailand). During the experiment sessions, the exact amount of toothpaste and volume of deionized water (NANOpure Ultrapure water system model D4745, Barnstead, Thermolyne, IA, USA) were measured by the investigators.

Before the brushing intervention, participants rinsed with 10 ml deionized water for 10 s and then unstimulated salivary F was collected as a salivary F baseline 5 min thereafter. After single brushing with F dentifrice and post-brushing rinse, the unstimulated whole saliva was collected. The researcher collected unstimulated saliva samples from each subject by collecting expectorated saliva into a container at the following time points: 0, 5, 10, 30, 60 and 90 min. If the collected saliva was not used in the same day of collection, it was stored at 4°C in a refrigerator. The collected saliva samples were centrifuged at 14,000 rpm for 5 min. The supernatant was used for further measurements.

Fluoride measurement

The 300 μl of saliva sample was mixed with 30 μl of total ionic strength adjustment buffer III to obtain 10:1 ratio (TISAB III, Thermo Electron Cooperation, Beverly MA, USA). The salivary F level (ppm) of each sample was measured using a calibrated ion-specific

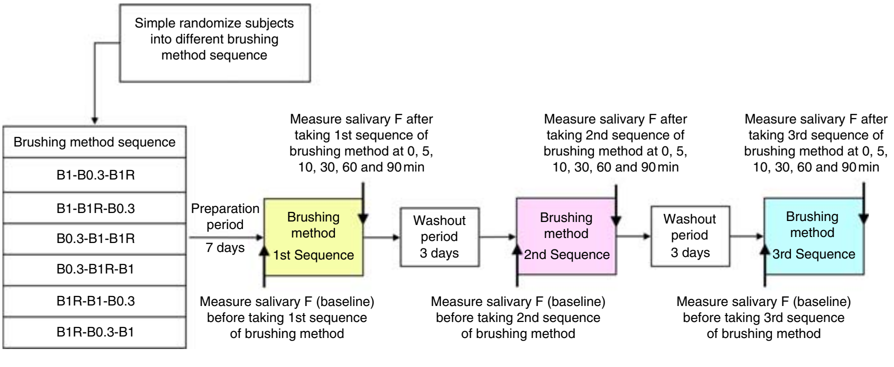


Figure 1.
The experimental crossover design study

electrode (ORION EA940, Thermoscienctific, Waltham, MA, USA) three times by a blinded investigator and reported as mean and standard deviation. Then, the results were used to plot the F concentration over the time curve and the salivary flow rate over the time curve.

Curve fitting

A F concentration curve was fitted using a graphing computer program (Kaleidagraph version 4.1.3, Synergy Software, Reading, PA) using non-linear regression procedures to the function[17] in the following equation:

$$F_s = A\exp(-\alpha t) + B\exp(-\beta t), \tag{1}$$

where F_s is the salivary F concentration at time t, A, B, α and β are parameters. A and B are intercepts at time 0, and α and β are elimination rates, of rapid phase and slow phase, respectively. Equation (1) is a two-compartment pharmacokinetic model to analyze drug clearance.

Area under curve (AUC) calculation

AUC is a bioavailability which is the amount of soluble F absorbed into the mouth. Each salivary F concentration curve's AUC was calculated by the following equation:

$$\mathrm{AUC} = \int_{t1}^{t2} F_s \mathrm{dt}, \tag{2}$$

the $t1$ and $t2$ of the rapid phase were 0 and 10, and those of the slow phase were 10 and 90 min, respectively.

Half-life ($t_{1/2}$) calculation

The half-life describes the amount of time required for the salivary F concentration to decline to half compared to its initial concentration. The half-lives of each elimination phase were calculated using the following equations:

$$\text{Initial } t_{1/2} = 0.693/\alpha, \tag{3}$$

$$\text{Late } t_{1/2} = 0.693/\beta. \tag{4}$$

Salivary flow rate measurement

The salivary flow rate was measured as described in the Navazesh protocol[18]. Briefly, the subject rested at least 5 min before saliva collection. The subject spat saliva in the mouth before collection. The subject then tilted his/her head forward, eyes opened and let saliva drop into a plastic container. The plastic container was pre-weighed before saliva collection. The unstimulated whole saliva was collected in a pre-weighed plastic container for 5 min. After collection, the container with saliva was weighed. The weight of saliva was the subtraction of weight of the container after and before saliva collection. Because more than 95 percent of saliva is water, 1 g of saliva equaled 1 ml. The salivary flow rate was calculated by the following formula:

$$\text{Salivary flow rate } (\text{ml/min}) = \text{container weight difference/time.}$$

Statistical analysis

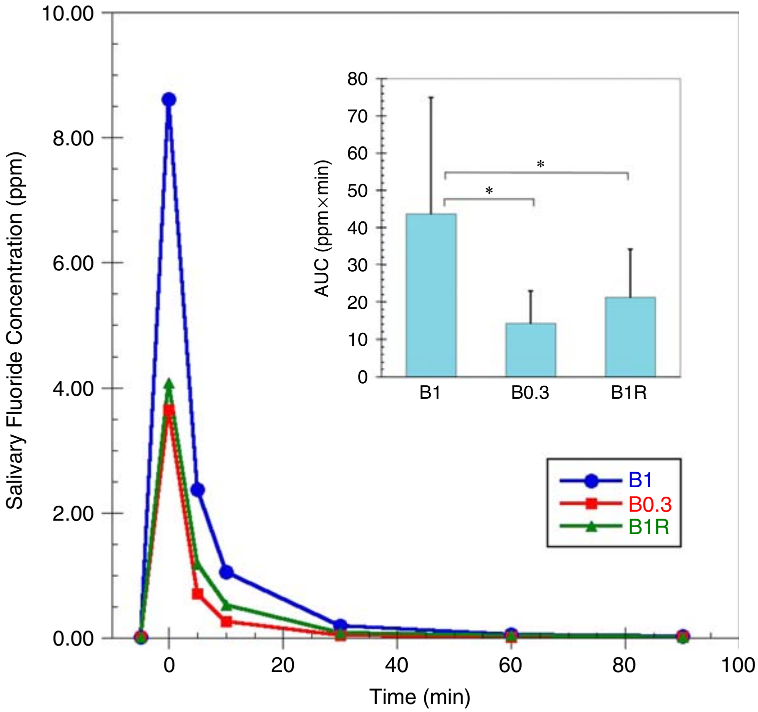
Repeated measures two-way ANOVA was used to analyze salivary flow rate and salivary F concentration at different time points. The $t_{1/2}$ and AUC were analyzed by one-way ANOVA. The F concentration was reported as mean and standard deviation. The nominal p-value of 0.05 was used to indicate significance. All results were analyzed with IBM SPSS Statistics version 22.0 (IBM Corporation, Armonk, NY).

Results

The baseline saliva F concentrations and salivary flow rate between the groups were not significantly different ($p = 0.063$ and $p = 0.959$, respectively). The interaction of intervention, period and sequence between brushing methods were not statistically different ($p = 0.852$). Thus, there were no carryover effects from the previous procedure.

Fluoride concentration and fluoride bioavailability

F concentration after brushing and rinsing procedures were dramatically decreased during the first 10 min then gradually decreased until reaching the baseline concentration at 90 min in all brushing procedures (Figure 2). The salivary F concentration at each time point for the B1 group was significantly higher compared with the B0.3 and B1 groups ($p < 0.001$), but B0.3 group was not significantly different from the B1R group ($p = 0.174$). The B1 and B1R groups exhibited a F level promoting remineralization, 0.04 ppm[3], for 60 min, while the B0.3 group retained this level for 30 min (Table I). The AUC represents the amount of soluble F (F bioavailability) from 0 min to each specific time point of measuring as the amount of F in the mouth. The AUC of B1 was significantly higher than B0.3 ($p < 0.001$) and B1R ($p = 0.002$) and there was no significant difference between B0.3 and B1R groups ($p = 0.777$).



Notes: The inset shows the area under the curve (AUC) of F concentration in the oral cavity of the B1, B0.3 and B1R groups. *Statistically significant at $p<0.05$

Figure 2. Mean salivary fluoride concentration decreased with time after brushing with 1,000 ppm *F* toothpaste and following post-brushing rinse procedures; B1 and B0.3 (brushing with 1 g and 0.3 g toothpaste without rinsing, respectively) and B1R (brushing with 1 g toothpaste then rinsing with 5 ml water for 10 seconds)

Time	Brushing method	Fluoride concentration (ppm) (mean ± SD)	Mean difference (mean ± SD) (p-value) B1	B0.3
Baseline	B1	0.016 ± 0.002		
	B0.3	0.012 ± 0.001	0.004 ± 0.002 ($p = 0.264$)	
	B1R	0.013 ± 0.001	0.003 ± 0.002 ($p = 0.446$)	0.001 ± 0.001 ($p = 1.000$)
0 min	B1	8.608 ± 1.161		
	B0.3	3.446 ± 0.476	5.162 ± 1.050* ($p < 0.001$)	
	B1R	3.939 ± 0.416	4.668 ± 0.987* ($p < 0.001$)	0.494 ± 0.452 ($p = 0.865$)
5 min	B1	2.165 ± 0.319		
	B0.3	0.673 ± 0.101	1.492 ± 0.291* ($p < 0.001$)	
	B1R	1.109 ± 0.134	1.057 ± 0.234* ($p = 0.001$)	0.436 ± 0.126 ($p = 0.264$)
10 min	B1	0.931 ± 0.148		
	B0.3	0.254 ± 0.039	0.677 ± 0.136* ($p < 0.001$)	
	B1R	0.494 ± 0.080	0.437 ± 0.118* ($p = 0.005$)	0.240 ± 0.063* ($p = 0.003$)
30 min	B1	0.166 ± 0.044		
	B0.3	0.051 ± 0.008	0.116 ± 0.041* ($p = 0.033$)	
	B1R	0.090 ± 0.017	0.076 ± 0.036 ($p = 0.145$)	0.040 ± 0.016 ($p = 0.063$)
60 min	B1	0.055 ± 0.010		
	B0.3	0.023 ± 0.003	0.032 ± 0.009* ($p = 0.009$)	
	B1R	0.036 ± 0.006	0.020 ± 0.010 ($p = 0.184$)	0.012 ± 0.006 ($p = 0.160$)
90 min	B1	0.028 ± 0.003		
	B0.3	0.015 ± 0.002	0.014 ± 0.002* ($p < 0.001$)	
	B1R	0.020 ± 0.003	0.008 ± 0.003* ($p = 0.017$)	0.006 ± 0.002* ($p = 0.023$)
	Brushing method	Fluoride bioavailability (ppm•min) (mean ± SD)	Mean difference (mean ± SD) (p-value) B1	B0.3
	B1	43.701 ± 31.177		
	B0.3	14.193 ± 8.684	29.509 ± 6.205* ($p < 0.001$)	
	B1R	21.263 ± 12.863	22.438 ± 6.205* ($p = 0.002$)	7.070 ± 6.205 ($p = 0.777$)

Notes: $n = 21$. *Statistically significant at $p < 0.05$

Table I.
Pairwise comparison of different measurements of fluoride concentration and fluoride availability in oral cavity

Half-life of fluoride in the oral cavity

The $t_{1/2}$ of F concentration occurred into two phases: the initial phase during which F rapidly decreased from 0 to 10 min and the late phase during which F slowly decreased at 30–90 min. The initial $t_{1/2}$ of B1 and B1R groups were significantly longer compared with the B0.3 group ($p = 0.042$ and $p = 0.001$, respectively), but there was no significant difference between the B1 and B1R groups ($p = 0.691$) (Table II). The late $t_{1/2}$ of the B0.3 group was significantly longer compared with the B1 group ($p = 0.015$) while that of the B1R group was not different from those of the B1 and B0.3 groups ($p = 0.724$ and $p = 0.268$, respectively) (Table II).

Half-life phase	Brushing method	Half-life (mean ± SD)	Mean difference (mean ± SD) (p-value) B1	B0.3
Initial	B1	3.124 ± 0.583		
	B0.3	2.680 ± 0.388	0.444 ± 0.175* ($p = 0.042$)	
	B1R	3.337 ± 0.675	0.212 ± 0.175 ($p = 0.691$)	0.656 ± 0.173* ($p = 0.001$)
Late	B1	3.124 ± 0.583		
	B0.3	2.680 ± 0.388	13.279 ± 4.562* ($p = 0.015$)	
	B1R	3.337 ± 0.675	5.398 ± 4.562 ($p = 0.724$)	7.880 ± 4.562 ($p = 0.268$)

Notes: $n = 21$. *Statistically significant at $p < 0.05$

Table II.
Pairwise comparison of different measurements of half-lives ($t_{1/2}$) of fluoride in oral cavity

Salivary flow rate
The salivary flow rate in all groups increased immediately after brushing and rinsing and then decreased to their rest stage within 30 min (Figure 3). There was no significant difference of salivary flow rate among the three groups ($p = 0.487$). This suggests that the amount of toothpaste and post-rinsing method had no effect on the salivary flow rate.

Discussion

F performs a major role in inhibiting demineralization[19]. When plaque pH is acidic, the CaF_2 from topical fluoride (stored in plaque) breaks down. These results in F penetration into enamel subsurface and adsorption to the surface of apatite crystals. F prevents the dissolution of the crystals. When the salivary pH becomes neutral, F is adsorbed to the surface of partially demineralized crystals and speeds up the growth of the new surface by bringing calcium and phosphate ions together and is also preferentially incorporated into the remineralized surface which is now more acid resistant[2, 20]. Chow and colleagues[21] found that low concentrations of F would promote remineralization. Lynch et al. performed *in vitro* studies by immersing tooth slices in various concentrations of F solution for three days. They reported that levels of 0.02 ppm F inhibited demineralization and 0.04 ppm F promoted remineralization in the pH-cycling study[3]. According to our study, the B0.3 group demonstrated an F concentration promoting remineralization for 30 min while the B1 and B1R groups maintained this level for 60 min. In our study, participants were assigned to brush only once. It would be interesting to know whether repeating the B1 brushing method could prolong F concentrations in the saliva.

The results of our study as well as other studies suggest that the salivary F retention is multi-factorial dependent on F concentrations, the amount of F in the oral cavity, salivary flow rates, the concentration of F in toothpaste, the rinsing method and F clearance (Figure 4) [7, 12, 13, 22].

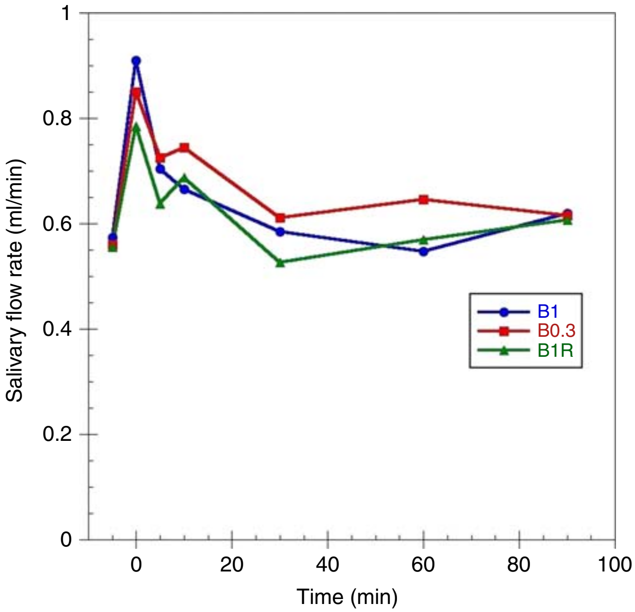


Figure 3.
Mean salivary flow rate at specific time points of B1, B0.3 and B1R groups

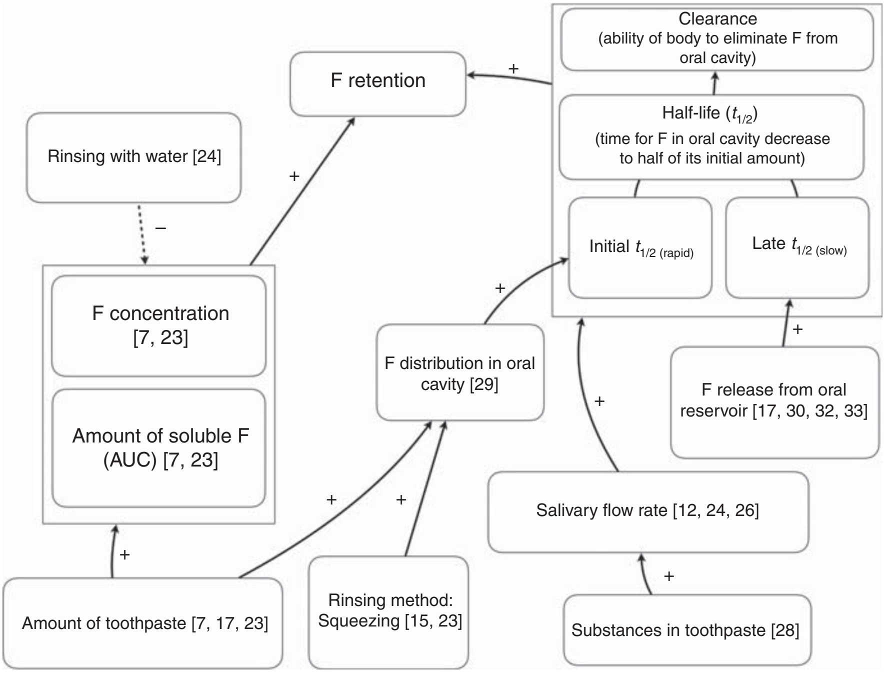


Figure 4.
Factors affecting fluoride retention

Notes: "F" is an abbreviation for fluoride. The solid arrows and plus sign (+) showed the direct effect of fluoride retention. The dash arrow and minus sign (–) showed the inverse effect of fluoride retention

The salivary F concentration at each time point and AUC of the B1 group were significantly higher compared to the other groups. This supports the view that the significant increase in F retention was correlated to a higher amount and non-rinsing of F toothpaste. The amount of F toothpaste played an important role in increasing the amount of F and bioavailability in the oral cavity[7, 23] and rinsing with water decreased both variables[24]. Tooth brushing without rinsing might be uncomfortable for some people so there was a recommendation for those people to rinse with F mouthwash to increase F concentration and bioavailability[25].

Oral F clearance is the term used to explain the removal of F from the oral cavity by swallowing, spitting and/or absorption[17]. It depends on salivary flow rates and $t_{1/2}$. The salivary flow rate is one of the key factors to maintain F retention in the oral cavity[26]. Immediately after brushing and rinsing with different interventions, each group showed the highest salivary flow rate and decreased to its rest stage within 30 min. This result was similar to studies using a F dentifrice[15], F mouthwash[12] or sugar[27]. The substances in F toothpaste such as flavor substances and menthol might stimulate salivary flow through smell and a cooling sensation[28]. The salivary flow rate has an inverse effect on $t_{1/2}$. The higher salivary flow rate will increase the dilution of F in the mouth and the frequency of swallowing which shortens $t_{1/2}$. However, there was no significant difference in flow rate between the three groups so there was no effect of the salivary flow rate on F clearance. We further investigated the $t_{1/2}$ and found that it divided into two phases: initial phase and late phase. This result was similar to previous studies referred to as the two-compartment open pharmacokinetics model[17]. In the initial phase, F concentration increased from the baseline to the highest concentration at time 0 then dramatically decreased within 10 min.

Saliva flushed F from the oral cavity by swallowing which was due to the elevated salivary flow rate[12, 24]. Because the F concentration and AUC of B1R and B0.3 displayed no significant difference, the $t_{1/2}$ of B1R and B0.3 group should not be different. But the B1R group had a longer initial $t_{1/2}$ compared with the B0.3 group, thus, the slurry-rinse method in the B1R group might provide a good distribution of F affecting initial $t_{1/2}$. The rinsing method by swishing F slurry of B1R group could distribute and store F to the enamel, interproximal space, labial and buccal vestibules, served as F reservoir[29]. Sjogren and Birkhed[15] suggested that this slurry-rinse method may be favorable in remineralizing enamel at approximal sites. Furthermore, the F in toothpaste, which was more viscous than NaF solution, penetrates slower through the plaque biofilm[30]. This implied that a small amount of water rinsing liquefied the toothpaste slurry, so it distributes through the oral reservoir. Zero *et al.*[23] stated that increasing contact time of F-containing slurry with the oral tissues resulted in greater F deposition. Besides, the muscular action of tongue and cheeks (during swishing) had a role in retaining F in the oral cavity[12]. The late phase, F concentration was slowly decreased due to the slow release of F from dental plaque and oral mucosal storage (oral reservoir) which affected the F retention[24, 30–33].

According to the WHO oral health report, dental caries are a major dental problem in Asian countries[1]. Various approaches to the effective use of fluoride have been investigated and developed. The results from our study revealed that the most effective brushing and post-rinsing procedure was to brush teeth with 1 g toothpaste with and without water as well as follow a post-rinsing procedure in order to prolong a remineralized F level for 60 min.

Limitation of the study

In this study, inclusion criteria (age, number of teeth, salivary flow rate) were controlled which may not directly apply to the community.

Possible clinical application

For dental caries prevention and the most efficient use of fluoride toothpaste, we recommend tooth brushing with 1 g of fluoride toothpaste for at least 2 min, and then expectorate excessive toothpaste slurry in the mouth without rinsing out with water. For people who feel uncomfortable about not rinsing, rinsing with fluoride mouthwash instead of water is recommended. For people who cannot calculate the precise amount of toothpaste to use on a daily basis, we suggest squeezing out an amount of F toothpaste that covers the full brush head. To prevent fluorosis and reduce the risk of excessive F ingestion for children, 0.3 g or a pea-sized amount of toothpaste is recommended for three- to six-year-old children that could raise the F level to the remineralizing level for up to 30 min[34]. However, the retention of F in children might be longer due to the slower flow rate compared to adults[35]. To apply our findings to children, especially – three- to six-year olds, further study should be conducted in this age group to ascertain that these three methods of brushing have similar levels of F retention in their oral cavities.

Conclusion

Non-rinsing and the amount of fluoride toothpaste used play an important role in raising salivary F and prolonging remineralizing levels.

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