Salivary fluoride concentrations following toothbrushing with experimental toothpaste containing S-PRG filler

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Research Article

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Abstract

Background: Single-blind, nine case comparative studies were conducted to evaluate salivary fluoride concentrations following toothbrushing using experimental toothpastes containing Surface Pre-Reacted Glass-ionomer (S-PRG) fillers. Preliminary tests were conducted in order to determine the volume of usage as well as the concentrations (wt%) of S-PRG filler. Based on the results given these experiments, we compared the salivary fluoride concentrations following toothbrushing with 0.5 g of 4 different types of toothpastes: 5 wt % S-PRG filler, 1400 ppm F AmF (amine fluoride), 1500 ppm F NaF (sodium fluoride), and MFP (monofluorophosphate) containing toothpaste.

Methods: Of the 12 participants, 7 participated in the preliminary study and 8 participated in the main study. All participants brushed their teeth using the scrubbing method for 2 minutes. At first 1.0 and 0.5 g of 20 wt % S-PRG filler toothpastes were used to compare, then followed by 0.5 g of 0 (control), 1, and 5 wt % S-PRG toothpastes, respectively. The participants spat out once and rinsed with 15 mL of distilled water for 5 seconds. Saliva was collected for 3 minutes each at different time intervals of 0 (baseline), 5, 10, 15, 30, 60, 120, and 180 minutes (min) after the rinsing. Fluoride concentrations were determined using a fluoride-electrode, and the area under the curve (AUC: ppm min) of each toothpaste was calculated as the salivary fluoride retention. The main study was then conducted to evaluate the salivary fluoride concentrations as well as the AUC value using 0.5 g of 5 wt % S-PRG filler toothpaste, followed by NaF, MFP, and AmF toothpaste.

Results: Since there were no statistical differences between using 1.0 g and 0.5 g of 20 wt % S-PRG toothpastes in salivary fluoride concentrations as well as the AUC value throughout the 180 min measurement, the volume was set as 0.5 g for the following studies. Concentrations of 5 and 20 wt % S-PRG toothpastes retained 0.09 ppm F or more in saliva even after 180 min. No statistical differences were seen in the salivary fluoride concentrations at any time intervals as well as the AUC value between 5 and 20 wt % S-PRG toothpastes. Based on these results, the concentration of 5 wt % S-PRG toothpaste was used for the main comparative study. MFP toothpaste resulted in by far the lowest salivary fluoride concentrations (0.06 ppm F at 180 min) and the AUC value (24.6 ppm min), whereas 5 wt % S-PRG toothpaste (0.15 ppm F at 180 min, 92.3 ppm min) displayed retention on par with AmF toothpaste which appeared to result in higher values (0.17 ppm F at 180 min, 103 ppm min), compared to NaF toothpaste (0.12 ppm F at 180 min, 49.3 ppm min).

Conclusions: The salivary fluoride concentrations following toothbrushing with 0.5 g of 5 wt % S-PRG filler containing toothpaste showed retention similar to the best performing 1400 ppm F AmF toothpaste even 180 min after toothbrushing.

Introduction

Anti-caries effects of fluoride (F) are related to the continuous presence of relatively low concentrations of F ion in the oral environment, surrounding the tooth surface. It has been reported that at least 0.04 ppm F
concentration in saliva can inhibit demineralization and enhance remineralization of the tooth [1,2]. However, salivary F\(^{-}\) concentration is usually only around 0.02 ppm in low water fluoridation areas [3]. Thus, retaining a higher F\(^{-}\) concentration of free F\(^{-}\) in the saliva is important to prevent the dissolution of the tooth surface. Toothbrushing with fluoridated dentifrices used daily is the easiest and most logical way to deliver and preserve F in the mouth [4-7]. Presently, many kinds of F toothpaste are manufactured using various F compounds. The most commonly used F compounds in toothpaste worldwide are Sodium fluoride (NaF) and Monouorophosphate (MFP). Toothpaste containing NaF immediately provides free F\(^{-}\) in saliva, however, toothpaste containing MFP provides the MFP ion together with some free F. According to the study conducted by Bruun et al., about 90% of the total F concentrations in NaF dentifrices were present as F\(^{-}\), whereas less than 10% of total F was F\(^{-}\) in MFP dentifrices [8]. Toda and Featherstone demonstrated that the concentrations of free F\(^{-}\) in 1000 ppm F MFP dentifrices diluted with double-deionized water showed only around 30-40 ppm, while 1100 ppm F NaF dentifrices showed around 1000-1100 ppm, respectively [9]. This is one of the reasons why F\(^{-}\) concentration in saliva following toothbrushing with NaF dentifrices showed not only significantly higher F\(^{-}\) concentration, but also more prolonged salivary retention than MFP dentifrices [10-12]. Thus, it is important for free F\(^{-}\) to be supplied to the oral cavity in relatively high concentrations (more than 0.04 ppm F in saliva) over long periods.

Recently, surface pre-reacted glass-ionomer (S-PRG) filler has attracted much attention as a bioactive functional glass filler, which releases six types of ions: BO\(_3^{3-}\), Na\(^+\), Al\(^{3+}\), SiO\(_3^{2-}\), Sr\(^{2+}\) and F\(^{-}\). S-PRG filler is a 3-layered bioactive glass filler. The proprietary multifunctional glass filler (glass core, the innermost layer) is coated with porous silica glass to create the surface-modified layer (the outermost layer). Then it is reacted with an aqueous solution of polyacrylic acid that results in a stabilized glass ionomer phase (the middle layer) on the surface of the multifunctional glass core. This glass-ionomer phase is responsible for the release of six types of ions as mentioned earlier [13,14]. The released ions can exhibit a variety of bioactive behaviors, such as preventing fungal [15,16] as well as bacterial adhesion [17-20], antibacterial activity [17-23], inhibiting demineralization of tooth enamel [24-27] and dentine [28-30], dentine remineralization [31] and formation [32], preventing bone loss [33], acid buffering ability [13,26], anti-plaque forming properties [17,19,21-23], and so on. Currently, S-PRG filler is often found in various dental products; resin-based composites [17,18], fissure sealants [26], tooth surface coating materials [24,25,29], resin adhesives [28], denture base-resin [34], tissue conditioner [16], temporary cement [30], orthodontic resins [35], polishing paste [36,37], etc. Along similar lines, S-PRG also has the potential to be an effective filler material for toothpaste. The use of S-PRG as a toothpaste filler represents a relatively new area for investigation in dental caries prophylaxis. Several bioactive effects have been reported due to the release of multi-ions from S-PRG containing toothpaste [38-45]. Amaechi et al. evaluated the effectiveness of toothpastes containing S-PRG filler in preventing tooth demineralization using human tooth enamel blocks by means of microbial caries model [39], and pH-cycling caries model [42] They concluded that 1 wt % or more S-PRG filler containing toothpastes inhibited tooth demineralization relative to the control set as 0 wt% S-PRG toothpaste [39] as well as 1100 ppm F NaF toothpaste [42], respectively. Similar
results of S-PRG filler containing toothpastes with respect to preventing tooth mineral loss were obtained from the studies conducted by Nakamura et al. [41] and Tomiyama et al. [43]. While Iijima et al. reported that in a comparison of a fourfold diluted solution of 950 ppm F NaF containing toothpaste and 5 wt % as well as 30 wt % S-PRG filler-containing experimental toothpaste (n=4 each), similar amounts of F⁻ were detected (NaF: 325.5 ppm F, 5 wt % s-PRG: 383 ppm F, 30 wt %: 234.5 ppm F) after 24 h [38]. However, there are no studies about the pharmacokinetics of S-PRG filler containing toothpaste in situ such as salivary F⁻ concentrations following toothbrushing with S-PRG filler toothpaste. Since not only salivary F⁻ concentration but also salivary F retention are the indicators of F availability in the fluid phase around the tooth, the estimation of these parameters is relevant, especially regarding new products based on different F concentrations [46]. Therefore, in this research, we have evaluated salivary F⁻ concentration and F retention following toothbrushing using S-PRG filler containing experimental toothpastes in two consecutive studies; clinical pre-testing and the main experiment. The preliminary test aims to determine the volume of usage (g) as well as the concentrations (wt %) of S-PRG filler for toothbrushing. Based on the results, we compared the salivary fluoride concentrations following toothbrushing with 0.5 g of 5 wt % S-PRG filler toothpaste and the other 3 different types of commercially available toothpastes, 1400 ppm F AmF (Amine Fluoride), 1500 ppm F NaF (Sodium Fluoride) and MFP (Monofluorophosphate) in the main study. It is important to mention that since the authors are testing new experimental toothpastes containing S-PRG filler, the production of such a prototype requires an industry partner. In this case, the partner is SHOFU Inc., Kyoto, Japan who provided the S-PRG filler containing experimental toothpastes to the authors for conducting the research and verified the information related to the test products.

Materials And Methods

Subjects

Initially, 16 participants were assessed for eligibility, of which, 4 participants were found to be ineligible (Fig. 1). Twelve healthy consenting adult volunteers (6 males, 6 females, aged 26 - 51 years) participated in this study. They had good general and oral health, natural dentition, as well a normal salivary flow rate. However, 5 participants in the clinical pre-testing and 4 participants in the main experiment could not participate completely, so their data were excluded/disqualified from aggregation in each study as necessary. One week before the pre-testing and the main experiments, the participants’ teeth were cleaned professionally and they were advised not to use any fluoridated products throughout the study except for between the 2 studies. The only allowance was non-fluoride toothpaste (Etiquette lion Aa, Lion company, Tokyo, Japan) on the toothbrush (DENT. MAXIMA, Medium-soft, Lion company, Tokyo, Japan). The subjects refrained from tooth brushing, eating, or drinking on the morning of each day of the experiment.

Materials (Toothpaste)

0 (control), 1, 5 and 20 wt % S-PRG filler, an average particle size of 1 μm diameter, containing toothpastes (SHOFU Inc., Kyoto, Japan) including hydrated silica, carboxymethylcellulose, sodium, glycerol, sorbitol, sodium lauryl sulfate, lime mint flavor, were used in the clinical pre-testing. Based on the
results, we used 5 wt % S-PRG filler containing experimental toothpaste to compare 1500 ppm F of NaF (Crest cavity protection, Procter & Gamble, Cincinnati, OH, USA) and MFP (Colgate cavity protection, Colgate-Palmolive Company, Dublin, Ireland), 1400 ppm F of AmF (Elmex junior, Colgate-Palmolive Company, Budapest, Hungary) in the main study. The ingredients of each toothpaste were shown in Table 1. Those commercially available toothpastes were of the same color and mint flavor in addition to almost similar F concentrations. The information in Table 1 is available in the public domain, including sources like Toothpaste packaging and respective websites.

Methods

Toothbrushing with fluoride toothpastes

Single-blind 5 case studies in the preliminary and 4 case studies in the main studies were carried out in this research. Prior to the experiment, no eating or drinking, as well as oral hygiene were confirmed. Resting saliva samples were collected into pre-weighed polypropylene centrifuge tubes (50 mL, Iwaki, Tokyo, Japan) for 5 min as a baseline. Individuals brushed their teeth in the same manner using the scrubbing method with a toothbrush (DENT. MAXIMA, Medium-soft, Lion company, Tokyo, Japan) for 2 minutes. The participants spat out once and rinsed with 15 mL of distilled water for 5 seconds. Saliva was collected for 3 minutes each at different time intervals of 0 (baseline), 5, 10, 15, 30, 60, 120, and 180 min after the rinsing (Fig 2). The toothbrush and each salivary sample tube were immediately weighed. Then the salivary flow rate (mL/min) was calculated. Subjects were allocated different toothpastes during each visit, in the following order, 20 wt % of 1 g and 0.5 g, then 0.5 g of 0 wt %, 1 wt %, 5 wt % S-PRG filler containing toothpastes in the clinical pre-testing, while 5 wt % of S-PRG filler, NaF, MFP, and AmF toothpastes with the same weight of 0.5 g in the main study. There was an interval of at least 1 week between the experiments.

F\(^-\) determination and calculation of the area under the curve (AUC) of F\(^-\) concentrations

One-tenth volumes of low-level TISAB (Total ionic strength adjustment buffer) were added to the saliva samples in addition to spitting-out as well as rinsing samples, and then F\(^-\) concentrations were determined using an F ion-specific electrode (Orion-9609, Thermo Fisher Scientific Inc., Chelmsford, MA, USA). The salivary F clearance curve (F-curve) was obtained from the results of F\(^-\) concentrations each time interval when plotting the data with time along the horizontal axis and F\(^-\) concentration along the vertical axis. The AUC of F-curve was calculated by trapezoidal rule, using a graph on which the horizontal baseline represents sampling time and the vertical scale represents F\(^-\) concentration (ppm min). Based on the study conducted by Creeth et al., the AUC was examined in the following three parts. AUC\(_{0-30}\) is during the first 30 min after the rinsing, AUC\(_{30-180}\) is the next term from 30 min to 180 min after the rinsing, and AUC\(_{0-180}\) is the entire period of time [47].

Statistical analysis
Statistical analysis of comparisons between F⁻ concentrations [ppm, mean ± standard error (SE)] in resting saliva (baseline) and salivary F⁻ concentrations following toothbrushing at each experimental time interval was carried out using the Wilcoxon signed-ranks test. F⁻ concentrations at each time point as well as the AUC value between 0.5 g and 1.0 g of 20 wt % S-PRG containing toothpaste were carried out using the Wilcoxon signed-ranks test.

The Kruskal-Wallis test and Bonferroni’s multiple comparison test were used to compare the salivary F⁻ concentrations at each time interval as well as the AUC value among the same volume (0.5 g) of 4 different concentrations (0, 1, 5, 20 wt %) S-PRG filler containing toothpaste. The same statistical analysis was used to compare 4 different types of F toothpastes (5 wt % S-PRG, NaF, MFP, AmF) in the main study. The statistical analysis software, IBM SPSS Statistics Ver.19 (IBM SPSS Japan, Tokyo) was used.

**Ethical considerations**

This study was conducted according to the principles of the Declaration of Helsinki on clinical studies with humans. The following ethical considerations were implemented when conducting this study. The research content was approved by the Health Sciences University of Hokkaido Hospital Ethics Review Committee (approval number 2015-027). In addition, we gave the appropriate education and knowledge to the volunteers in advance that they could withdraw at any time of their own will and that there would be no consequences after their withdrawal. The informed written consent was obtained from all participants.

**Results**

**Preliminary-testing**

Regarding the baseline of salivary flow rate, there were no statistically significant differences among the experiments, for which the average value was 0.41 ± 0.06 mL/min. The average salivary F⁻ concentration of baseline was 0.021 ± 0.004 ppm, and no statistical differences were seen among the experiments.

Table 2 shows the comparison of salivary F⁻ concentrations in each time interval when different amounts (0.5 g and 1.0 g) of 20 wt % S-PRG containing toothpastes were applied. Fig 2 shows F-curves obtained from the results of Table 2. No significant differences in F⁻ concentrations were observed when applying 0.5 g versus 1.0 g, except for the measurements taken 15 min following toothbrushing. Table 3 shows the comparisons of the AUC value between 0.5 g and 1.0 g of 20 wt % S-PRG containing toothpastes. Only AUC0-30min of 0.5 g applied was significantly higher than 1.0 g (p<0.05).

Table 4 shows the comparisons of salivary F⁻ concentrations following toothbrushing with 0.5 g of 4 different concentrations (0, 1, 5, 20 wt %) of S-PRG toothpastes and Fig 3 shows each F-curve. Although 0 wt % toothpaste showed no significant differences in F⁻ concentrations from the baseline over time, all
time intervals of 1, 5, and 20 wt % toothpastes displayed significantly higher levels of F- concentrations than the baseline (p<0.05). The F-curves of 1, 5, and 20 wt % S-PRG toothpastes show that the highest F- concentrations were observed 5 min after rinsing, and F- concentrations retained above 0.04 ppm during 120 min. Moreover, 5 and 20 wt % toothpastes sustained over 0.09 ppm even beyond 180 min after the rinsing. The 20 wt % toothpaste was the highest F- concentrations, whereas the 1 wt % was the lowest F- concentration among 1, 5 and 20 wt % S-PRG toothpastes in each time interval, except for 5 wt % at 5 min after rinsing. At any given time interval, there were no significant differences in salivary F- concentrations between toothpaste containing 5 wt % and 20 wt % S-PRG filler. Table 5 shows the comparisons of AUC (ppm min ± SE) values from each F-curve. There were no significant differences between 5 wt % and 20 wt % at any terms. 1, 5, and 20 wt % S-PRG toothpaste showed significant differences when compared with the control experiment (0 wt %).

**The main study**

At the baseline, the mean salivary flow rate was 0.57 ± 0.06 mL/min, and the mean salivary F- concentration was 0.016 ± 0.002 ppm. No statistically significant differences were seen among 4 experiments.

Table 6 shows the F- concentrations of spitting-out and rinsing samples after toothbrushing with 4 different types of toothpastes. S-PRG was the by far lowest, but there was no significance between S-PRG and MFP toothpastes in spitting-out samples. However, significantly, MFP toothpaste was lowest in rinsing samples (p<0.01). Table 7 shows the salivary F- concentrations following toothbrushing with 0.5 g of 5 wt % S-PRG, NaF, MFP, and AmF toothpaste. Fig 4 shows F-curves resulting from Table 7. All time intervals of any type of toothpastes displayed significantly higher levels of F- concentrations than the baseline (p<0.05). MFP, which resulted in the lowest salivary concentrations of F- over the entire period of time, showed significant differences in comparison with AmF, as well as S-PRG throughout the study. A significant difference between MFP and NaF was seen in the measurements taken 5 min after toothbrushing (p<0.05). Table 8 shows the comparisons of AUC values from each F-curve. MFP toothpaste showed significantly lower values than any other toothpaste in AUC0-180min. In AUC0-30min and AUC30-180min, there were significant differences between MFP and AmF, as well as MFP and S-PRG, respectively (p<0.01). Among S-PRG, NaF, and AmF toothpastes, no significant differences were observed any terms of AUC.

**Discussion**

In recent years, the role of toothpaste has been changing from tooth cleaning auxiliary agents to essential products, to meet various needs such as prevention of dental caries, periodontal disease, halitosis, coping with hypersensitivity, esthetics, and so on [48]. Toothpaste as a quasi-drugs can easily deliver those various effective medical ingredients into the oral cavity. During toothbrushing with F toothpaste, the F diffuses into the important reservoir of F in the mouth which consists of saliva, teeth, plaque, and oral
mucosa. Such F has a significant positive role in dental caries prevention. When F exists in the oral cavity, the OH in Hydroxyapatite \( \text{HAP}: \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) in tooth crystals is replaced by F. As a result, the Hydroxyapatite \( \text{HAP}: \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) becomes Fluoroapatite \( \text{FAP}: \text{Ca}_{10}(\text{PO}_4)_6(F)_2 \). The F thus created, has been clinically proven to exert an anti-caries effect by increasing the acid resistance of the tooth surface. However, according to Featherstone, even if F does not necessarily replace an (OH) part in the tooth crystal of HAP, it is important that 0.04 ppm or more \( F^- \) cover the tooth surface for a long time or exist continuously on the periphery of the tooth crystal. This phenomenon results in the prevention of tooth demineralization [1]. Thus, in order to get prolonged F retention in saliva, many kinds of F toothpaste, such as different F concentrations, different F compounds, and different forms of toothpaste are being manufactured by companies worldwide. To avoid negative side effects such as F poisoning or fluorosis, etc., the maximum concentration of F used in toothpaste is limited to 1500 ppm [48]. Globally, NaF and MFP are the most common F compounds used in commercial dental hygiene products [48]. A critical review concluded that there are statistically significant differences between the anti-caries effectiveness of toothpaste containing NaF and MFP respectively [49], due to their differences in F bioavailable processes in the oral cavity. Toothpaste containing NaF immediately provides free ionic F in saliva. However, toothpaste containing MFP provides the MFP ion together with some free F\(^-\). The hydrolysis of the MFP ion to produce free F\(^-\) is positively influenced by enzymes such as alkaline phosphatase in oral fluid (saliva, plaque, bacteria, and enamel). The aforementioned enzymes can aid the hydrolysis process gradually in the mouth [49-52]. Moreover, MFP toothpaste is less stable than NaF, the capacity to release F in the case of MFP toothpaste (fresh or old) decreases with time depending on storage conditions (like humidity, temperature, etc.) [53,54]. Thus, F toothpaste is needed to not only keep F stable for a long time but also to release and disperse F\(^-\) rapidly, thereby prolonging the retention of relatively high concentrations in saliva after usage. From this context, S-PRG toothpaste possessing the quality of releasing F\(^-\) quickly and over a long period of time has been developed by the Shofu Inc., Kyoto, Japan [38-45]. S-PRG technology allows for the formation of a stable glass-ionomer phase in fillers by pre-reacting acid-reactive glass-containing fluoride with polycarboxylic acid in the presence of water. S-PRG fillers also have the ability to release Al, B, F, Na, Si, and Sr ions [13,14]. Sr and F ions are known to be strong inducers of remineralization of teeth. Sr and F ions also improve the acid resistance of teeth by acting on hydroxyapatite by converting it to Strontiumapatite [55-58] and Fluoroapatite [2,56,58,59], respectively. However, there has been no investigation in the scientific domain so far about how much S-PRG filler toothpaste can release these respective ions and how long it can retain them in the oral cavity after toothbrushing. For this reason, salivary F concentrations and F retention following toothbrushing with S-PRG filler containing toothpaste for 180 min were evaluated. We compared the S-PRG filler toothpaste to NaF, MFP in addition to AmF toothpaste which is available as one of the OTC toothpaste in Europe. AmF has a hydrophobic non-polar tail with a hydrophilic polar amine head. As a consequence of this molecular structure, AmF acts as a surfactant and thus can form a protective film on oral surfaces. This protective film is resistant to dissolution by acid in saliva, there by strong evidence of tooth enamel dissolution [9,60-63]. Besides, the reduction of solubility of enamel by AmF toothpaste is better than inorganic F toothpaste. Therefore, AmF toothpaste was included in this study as a relevant
comparison for the S-PRG filler containing toothpaste. Out of the 12 participants, only 7 subjects were able to in all experiments. The design of this study meant that even with a washout period, the effect of the previous intervention may not have completely disappeared. However, there were no differences seen in the baseline of resting salivary F\textsuperscript{-} concentration in each experiment, so it seems to be insignificant. Only non-fluoridated toothpaste was allowed to use during experiments. This might have also helped the baseline of F\textsuperscript{-} concentrations to not have significant differences. As for toothbrushing methodology, the brushing manner used in this research was based on the recommendation by The Japanese Association for Dental Science (Fluoride Working Group) for adults [64] which modified the F toothbrushing technique by Sjögren et al. [65], except for the amount of usage. Salivary F\textsuperscript{-} concentration and F retention after toothbrushing with fluoridated dentifrices are affected by several factors in relation to a) salivary flow rates [10, 66-68], b) swallowing of saliva [10, 67], c) F concentrations and formulations in dentifrices [5-12, 46-54, 60-63, 69-75], d) amount of dentifrices usage [5, 6, 8, 10, 47, 65, 69, 76-78] along with a time required for tooth brushing [10, 47, 65, 69], e) amount of water and frequencies of mouth rinsing [47, 60, 63, 65, 66, 68-70, 76-78] and so on. Considering these factors, this F toothbrushing technique used has proven to be a simple and effective method for dental caries prevention. According to Duckworth et al., oral F levels depend on the dose of F concentrations [70], and oral F levels increase significantly with increasing F concentration [10]. Contrary to the expectations based on Duckworth et al. and others, 0.5 g differences in the amount of 20 wt % S-PRG filler toothpaste (i.e. 1 g vs 0.5 g) did not result in significant differences in each salivary F\textsuperscript{-} concentration. Although the conclusion from previous research suggests that 1 g of toothpaste is the appropriate amount for adults [64, 69], it was clear from our results that while using S-PRG containing toothpaste, 0.5 g of toothpaste is sufficient. In addition to cost-effectiveness, a smaller amount of toothpaste is easier to facilitate toothbrushing. So the remaining experiments of the study used toothpaste samples of 0.5 g. The result of this experiment is probably due to S-PRG having the property of continuously releasing ions. There may be an optimal amount of toothpaste to use for the limited amount of saliva in the oral cavity. Salivary F\textsuperscript{-} concentration may not change a lot when the ratio of saliva to toothpaste exceeds a certain value. As for the concentration of S-PRG filler (wt %), there were no significant differences between 5 and 20 wt % in salivary F\textsuperscript{-} concentrations at any time interval as well as F retention. One explanation for this finding is that S-PRG filler toothpaste has the ability to release cation ions including Na, Al, and Sr ions at the same time. Some of these ions may combine with F\textsuperscript{-} in saliva and then, become complex salts. With increasing concentrations of S-PRG filler containing toothpaste, more ions would be released, possibly resulting in more complex salt, so-called easily bound F, being formed as well. Such complexes may not only enhance F\textsuperscript{-} release but also contribute to a rapid release of F\textsuperscript{-} into saliva by means of forming soluble salt. Further research is needed on the behavior of ions released from S-PRG filler containing toothpaste in the oral cavity.

With regard to boundF, it can be confirmed by AUC which consists of 2 dimensions (ppm min). Creeth et al., who determined the F\textsuperscript{-} concentrations in saliva up to 120 min following toothbrushing with fluoridated toothpaste, used the area under the complete measured saliva clearance curve (i.e. AUC\textsubscript{5–120 min}) as the total F retained in the oral cavity following brushing and rinsing [47]. According to this definition, the total
F bound (bound F⁻) in the oral cavity following brushing and rinsing was estimated as the area under the measured saliva clearance curve after 30 minutes (i.e. AUC\textsubscript{30–120 min}). This allows a 30-minute period for any F retained but not bound in the oral cavity to be rinsed out by normal salivary flow [67, 70]. The difference between the ‘bound F⁻’ and ‘total F⁻’ pools is denoted as the ‘unbound F⁻’ pool (i.e. AUC\textsubscript{5–30 min}). Based on this research, we evaluated the salivary F retention of the AUC as divided into 3 phases: AUC\textsubscript{0–180 min} as total F, AUC\textsubscript{0–30 min} as unbound F, and AUC\textsubscript{30–180 min} as bound F, respectively. Easily rinsed out free F⁻ was seen in the term of the first 30 min, after that free F⁻ would be released from easily bound F gradually into saliva. It seems that total F retention (AUC\textsubscript{0–180 min}) will be strongly affected by unbound F⁻, i.e. free F⁻ (AUC\textsubscript{5–30 min}). In our results, there were no significant differences among 1, 5, and 20 wt % S-PRG toothpaste in AUC\textsubscript{30–180 min}. Here, in AUC\textsubscript{0–180 min} and AUC\textsubscript{0–30 min}, 1 wt % showed the lowest retention, while 5 wt % and 20 wt % showed no significant differences. Thus, there is a possibility of better salivary F retention in case of at least 1 wt % g of S-PRG filler containing toothpaste. Though 0.5 g of 5 wt % S-PRG toothpaste was used in this study, 1.0 g of 1 wt % S-PRG filler containing toothpaste might have high F retention as well as 0.5 g of 5 wt % S-PRG filler toothpaste after toothbrushing. There is room for further investigations on the optimal concentration and amount of usage with S-PRG filler containing toothpaste.

On the other hand, a comparable to the amount of F⁻ observed using NaF and AmF containing toothpaste was detected in saliva in addition to prolonged F retention following toothbrushing with 0.5 g of 5 wt % S-PRG filler containing toothpaste in this research. The F compound of NaF toothpaste is confirmed to be able to rapidly dissolve in saliva and release free F⁻. F⁻ spread immediately into the mouth, some of them are adsorbed to the oral tissues as a bound F. Consequently, salivary F concentration can maintain higher levels as mentioned before. While AmF toothpaste displayed the tendency of higher salivary F levels, in agreement with a previous study by Issa et al [60] and Albahrani et al [63]. They explained that AmF toothpaste may be due to its special molecular structure, in which the F⁻ is bound to an organic fatty acid amine fragment. This type of structure, a combination of a hydrophobic hydrocarbon chain with a hydrophilic head group, is typical for tensides, which are characterized by their surface activity; they accumulate systematically on surfaces of all kinds. Thus, AmF is reported to hold F in contract with tooth surface for longer periods and to reduce the solubility of enamel better than inorganic fluoride. Besides, it is recognized that it is associated with a reduction in dental plaque adhesiveness, due to the greater affinity of hydrophilic counter-ions to the enamel [79]. The rapid release of F ions from NaF and AmF toothpaste is clear in Table 6, which shows the high F⁻ concentrations in the spitting-out samples. The low F⁻ concentrations of S-PRG toothpaste in spitting-out samples may be due to the fact that S-PRG toothpaste does not contain F compounds as an F ion-releasing source. It is well known that high-concentrated fluoridated products such as topical application of F (around 10,000 ppm F) produce bound F such as CaF₂ or CaF₂-like deposits on the enamel surface which slowly releases F⁻. These deposits act as a pH-controlled F and calcium reservoir. However, there is no strong evidence for the formation of these CaF₂ or CaF₂-like deposits after brushing with fluoridated dentifrices [10]. According to Suge et al., the formation of CaF₂ was not detected on pH-cycling HAP pellets after being treated with any
concentration of S-PRG toothpaste measured by a ‘Powder X-ray Diffraction Analysis’ [44]. Fujimoto et al. demonstrated that ion release from S-PRG filler was influenced by the mixing ratio of the solution rather than the initial pH of the solution. F⁻ release was suppressed by the lower ratio (1000 g : 1 L 100 g : 1 L), whereas it significantly increased when the ratio was high (1 g : 1 L) in distilled water (pH 5.9) as well as in lactic acid solution (pH 3.8). Additionally, S-PRG filler had a modulation effect on acidic conditions, causing the pH of the surrounding environment to become weakly alkaline upon contact with water or acidic solutions [13]. It may be that in addition to the F concentrations of toothpastes, the pH of the oral environment also affects salivary F retention, by extension bound F⁻ formation, of the S-PRG filler containing toothpaste.

Several studies have shown that S-PRG-containing toothpaste can inhibit the demineralization of both tooth enamel and dentin in vitro. In relation to this, Nakamura et al. have demonstrated an experiment on bovine enamel blocks treated twice a day for 4 days by experimental pastes containing 0-30 wt % S-PRG filler. In their experiment using Micro CT and SEM examination methods, the lowest mineral loss and high concentration of Sr on the enamel surface were observed in 10 wt % S-PRG toothpaste [41]. Amaechi et al. investigated the mineral loss and the lesion depth of human enamel blocks treated with 0–30 wt % S-PRG filler containing toothpaste using the pH-cycling caries model. Furthermore, their study using quantitative light-induced fluorescence (QLF) and Transverse Microradiography (TMR) revealed that the S-PRG filler containing experimental pastes effectively inhibited demineralization of enamel surface compared to 1500 ppm F NaF toothpaste [42]. Shinkai et al., who investigated the effect of S-PRG paste (0-30 wt %) on the acid resistance of the enamel surface of the human tooth using pH-cycling, reported that each lesion's depth of enamel polished with 5, 20, and 30 wt % paste was significantly shallower than that polished with 0 wt % when analyzed by a polarized light microscope (PLM) [37]. Tomiyama et al. reported that 30 wt % S-PRG toothpaste inhibited bovine dentin demineralization markedly compared to NaF toothpaste [43]. Whether S-PRG filler containing toothpaste has an influence on the remineralization of the enamel surface/dentin has to be elucidated in further experimental and clinical studies.

Conclusion

Our study revealed that 5 wt % S-PRG filler containing toothpaste released F⁻ rapidly after toothbrushing when using 0.5 g toothbrushing and can retain 0.1 or more ppm F⁻ for 180 min, a level which is comparable to 1500 ppm F NaF and 1400 ppm F AmF containing toothpaste. In a recent systematic review of the literature, it was reported that dentifrices containing F concentrations of 1000 ppm F or more would only have cariostatic effects for children and adolescents [80], thus toothpaste containing at least 5 wt % S-PRG filler will be useful, and beneficial for dental caries prevention.

Declarations

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Abbreviations

F (fluoride), F⁻ (fluoride ion), NaF (sodium fluoride), MFP (monofluorophosphate), AMF (amine fluoride), S-PRG (surface pre-reacted glass-ionomer), AUC (The area under the curve)

Ethics approval and consent to participate

This study was conducted according to the principles of the Declaration of Helsinki on clinical studies with humans. It was approved by the Ethics Committee at Health Sciences University of Hokkaido Hospital Ethics Review Committee (approval number 2015-017). Every participant in this study signed informed consent before their inclusion.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Authors’ contributions

Conceptualization, Mina Hirose (M.H.); methodology, M.H.; investigation, M.H. and Yukie Murata (Y.M.); resources, M.H., M.Y., Atsushi Fukuda A.F., Shoko Yahata (S.Y.), Yusuke Fujita (Y.F.), Minako Kaji (M.K.) and Masato Saitoh (M. S.); writing – original draft preparation, M.H.; review and editing, M.H.; project administration, M.H. All authors have read and agreed to the published version of the manuscript.

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References


Tables

Tables are available in Supplementary Files section.
Figures

Figure 1  Experiment flow chart and participants selection

Figure 1

See image above for figure legend
Figure 2 F concentrations in saliva following toothbrushing with different applied amount of 20 wt % S-PRG containing toothpastes (n=7)

Figure 2

See image above for figure legend
**Figure 3**

See image above for figure legend.

**Figure 3** F concentrations in saliva following toothbrushing with different wt % of S-PRG filler containing toothpastes (n=7)
Figure 4 F concentrations in saliva following toothbrushing with 4 different types of F toothpastes (n=8)

Figure 4

See image above for figure legend

Supplementary Files

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