Research Article

Subgingival uptake and retention of stannous fluoride from dentifrice: Gingival crevicular fluid concentrations in sulci post-brushing

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Abstract: Purpose: To examine the delivery of stannous fluoride to subgingival sulci following toothpaste use in a clinical population. **Methods:** This was a controlled, single-site study. 23 subjects with at least 20 dental pockets, 2-4 mm with bleeding, who had not used a stannous fluoride dentifrice in the last 3 months were enrolled. After a 2-week washout period, 20 subjects returned for a baseline visit. They were instructed to refrain from brushing the night before the baseline visit. GCF samples were taken from up to 10 sites identified as sampling sites. Subjects were then given a 0.454% stannous fluoride dentifrice and soft manual toothbrush and asked to brush for 1 minute. 30 minutes after brushing, GCF was re-sampled. Subjects continued using the stannous fluoride dentifrice and soft manual toothbrush at home, twice daily for 2 weeks, in place of their usual hygiene products. At Days 1 and 14, subjects returned to the site, and 12 hours post-brushing GCF samples were taken. The samples were analyzed by ICP-MS (inductively coupled plasma mass spectrometry). A Wilcoxon signed-rank test was performed to determine the difference between post-baseline visits and baseline. Statistical tests were 2-sided using a 5% significance level. **Results:** 20 subjects completed the trial. Significant levels of tin, a marker for stannous fluoride, were detected 30 minutes after brushing at sampling sites of 2-4 mm. The median tin level in gingival crevicular fluid (GCF) was 24.59 ng/µl, which was highly significant versus baseline ($P< 0.0001$). Tin levels sampled in GCF 12 hours after brushing on Days 1 and 14 were highly significant versus Baseline ($P< 0.0001$), showing an increasing trend with continued use. (*Am J Dent* 2018;31:184-188).

Clinical significance: Stannous fluoride was found to penetrate sampling sites from 2-4 mm and was retained for 12 hours. Subgingival uptake and retention of stannous fluoride following toothbrushing may play a role in detoxification effects on microbial biofilms and may contribute to the therapeutic efficacy of stannous fluoride dentifrices in promoting gingival health.

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Introduction

Stannous fluoride has a long history of use for the prevention of dental caries, and was in fact the first clinically proven chemotherapeutic added to toothpaste in the 1950s.[1] Although commercial use of stannous fluoride declined in the 1980s, the last two decades have seen a renaissance in its use in dentifrices.[2-6] Today stannous fluoride formulations have gained widespread recognition for efficacy in improving the health of both hard and soft tissues. With respect to soft tissues, numerous studies have demonstrated that formulations containing stabilized stannous fluoride (Procter & Gamble) provide clinical benefits in reducing gingivitis.[7-16]

Our laboratories have undertaken extensive research to develop a further understanding of the mechanisms of stannous fluoride in promoting improved gingival health. Recently,[17] some properties of stannous fluoride which may contribute to efficacy in the prevention and treatment of gingival diseases were discovered, including the ability of stannous fluoride to alter anaerobic microbial metabolism of the microbiota and also interfere with microbial antigenicity through deactivation of both lipopolysaccharide (LPS) and lipoteichoic acid (LTA) with host pattern recognition receptors (toll receptors).[18-20] In clinical studies,[21,22] subgingival dental plaque sampled from subjects brushing with stannous fluoride dentifrice exhibited decreased endotoxin pathogenicity compared to dental plaques collected at baseline.

The detoxification of bacteria requires the direct interaction of stannous fluoride with resident bacteria at the site of periodontal infection, presumably in subgingival areas viz. the sulcus. While the retention of stannous fluoride in dental plaque and saliva following toothbrushing has been demonstrated,[23,24] no studies to our knowledge have examined the penetration and retention of topically applied stannous fluoride to subgingival sites.

The current clinical study explored the penetration and retention of stannous fluoride in gingival sulci following toothbrushing. Samples of gingival crevicular fluid were collected following toothbrushing with stannous fluoride dentifrice 30 minutes and 12 hours post toothbrushing after 1 day and 2 weeks' use of the dentifrice.

Materials and Methods

Study description and population

Study description - This was a 2-week controlled, patient-blinded single-site clinical study. Twenty subjects meeting entrance criteria were enrolled in a protocol including tooth brushing with an assigned stannous fluoride toothpaste and manual toothbrush. GCF and plaque were collected from subjects 30 minutes post-brushing at Baseline and 12 hours post-brushing on Day 1 and Day 14, with analytical assessments for tin uptake and retention in samples collected from selected sites. The study protocol was approved by the U.S. Institutional Review Board (U.S.IRB2017SRI/07; clinical trials.gov registration number: NCT03296657).

Study population - To qualify for the study, subjects had to provide written informed consent, be 18 years of age or older,

agree to all study requirements, and present with a minimum of 20 sampling sites without amalgam restorations which included bleeding on gentle probing and pocket depth ≥2 mm but not deeper than 4 mm. Subjects were excluded if they had used a stannous fluoride dentifrice in the last 3 months. Up to 10 sites were identified per subject as "sampling sites" for gingival crevicular fluid (GCF), which was the primary outcome and the focus of this report, as well as supragingival and subgingival plaque as secondary measures which are not reported here.

Study visits /Subject instructions/Sample collections

Visit 1 - Qualification and acclimation - Product distribution - At this visit, subjects read and signed an informed consent and personal medical history, demographic information and entrance criteria were obtained. A trained examiner performed an oral examination, a Gingival Bleeding Index (GBI) exam, and a Pocket Depth (PD) exam to determine eligibility. Subjects meeting the entrance criteria were given acclimation product with usage instructions. Prior to leaving the site, subjects were scheduled for their Baseline visit (approximately 2 weeks from Acclimation visit) and reminded to bring their study products back to their next study visit. Subjects were also reminded to refrain from brushing their teeth after 11 p.m. the night prior to their next visit and to refrain from eating, chewing gum, drinking (small sips of water were allowed up to 45 minutes prior to the visit), and using tobacco for 4 hours prior to their baseline visit. Subjects were provided with a kit box containing an overlabeled tube of regular sodium fluoride dentifrice (Crest Cavity Protection toothpaste[a]) for blinding purposes and an Oral-B Indicator[a] soft toothbrush for use twice daily in their customary manner during their 2-week acclimation period. Subjects were asked to refrain from flossing or using any other oral hygiene products for the duration of the 2-week acclimation period.

Visit 2 - Baseline and 30 minutes post brushing sampling - Prior to this visit, subjects had been brushing with regular (non-stannous fluoride) dentifrice for the 2-week acclimation period. Subjects were reminded, either by phone or by e-mail, to refrain from brushing their teeth and from performing any other oral hygiene procedures after 11 p.m. the night prior to the clinic visit and to refrain from eating, chewing gum, drinking, and using tobacco 4 hours prior to the visit. Continuance criteria were assessed and the same examiner performed an oral examination followed by the collection of samples from the pre-identified sampling sites including three supragingival plaque, three subgingival plaque, and two GCF samples. Sampling included up to eight baseline collections; supragingival and subgingival plaques were taken from the same sites; GCF samples were taken from different sites. Subjects then received a kit box with treatment products, including overlabeled 0.454% stannous fluoride dentifrice[a] for blinding purposes and an Oral-B Indicator soft toothbrush. Subjects were instructed to brush twice daily with provided products, applying at least a 1-inch strip of the product on the head of their toothbrush, brushing thoroughly for 1 minute and expectorating. Subjects could rinse their mouth with tap water following brushing. Subjects were asked to refrain from flossing or using any other oral hygiene products for the duration of the study.

Upon receipt of their test product, subjects were asked to brush in the clinic as instructed, supervised by the study staff. After 30 minutes, the examiner collected samples from a new set of comparable pre-identified sampling sites. After the second set of sample collections, subjects were scheduled for their 12-hour post brushing visit on Day 1. Subjects were reminded to refrain from brushing their teeth and from performing any other oral hygiene procedures 12 hours prior to the morning visit and to refrain from eating, chewing gum, drinking, and using tobacco 4 hours prior to the visit. All general comments and Adverse Events (AEs), if any, were recorded.

Visit 3 - Day 1 - 12-hour post-brushing sampling - Continuance criteria were assessed including confirmation of the last toothbrushing (at least 12 hours). Samples were collected from the pre-identified sampling sites. After the sample collection, subjects were scheduled for their Week 2 visit. Subjects were reminded to refrain from brushing their teeth and from performing any other oral hygiene procedures 12 hours prior to the visit and to refrain from eating, chewing gum, drinking, and using tobacco 4 hours prior to the visit. All general comments and AEs, if any, were recorded.

Visit 4 - Day 14 - 12-hour post-brushing sampling – Continuance criteria were assessed and the examiner repeated the oral examination followed by the collection of samples from the pre-identified sampling sites as described at other visits.

Clinical examinations and sampling

Oral examinations - At each clinic visit subjects received oral examinations primarily to evaluate potential AEs. These included professional visual examination of oral soft tissue of the oral cavity and perioral area utilizing a standard dental light, dental mirror, and gauze. The structures examined include the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips, and perioral area. Assessment of the oral hard tissues was conducted via a visual examination of the dentition and restorations utilizing a standard dental light, dental mirror, and air/water syringe. An AE was recorded if a new abnormal finding was noted after treatment application or any abnormal finding noted prior to treatment application increased in severity after treatment onset.

Gingival Bleeding Index - GBI assessments were carried out to qualify subjects for the study. A standard clinical method was applied.[25] With this method, the gingivae were lightly air-dried, after which a periodontal probe with a 0.5 mm diameter tip was inserted into the gingival crevice to a depth of 2 mm or until slight resistance was felt. The probe was then run gently around the tooth at an angle of approximately 60° and in contact with the sulcular epithelium. Minimum axial force was used to avoid undue penetration into the tissue and the probe was moved around the crevice, gently stretching the epithelium. Each of the three gingival areas, i.e., buccal, mesial/distal and lingual, of the teeth was probed in this manner waiting approximately 30 seconds before recording the number of gingival units that bled, using the following Gingival Bleeding Index criteria:[25]

Score	Criteria
0	Absence of bleeding after 30 seconds
1	Bleeding observed after 30 seconds
2	Immediate bleeding observed
8	Non-gradable site
9	Missing tooth

Probing pocket depth (PPD) - PPD was measured in subjects to qualify them for the study and to identify sampling sites. PPD was recorded in subjects by the study clinician using a UNC 15 mm probe[b]). The probe was inserted parallel to the root surface and directed apically toward the perceived location of the apex of the root until slight resistance was felt. PPD was assessed as the distance from the gingival margin to the apical end of the pocket measured in mm. Probe recordings were rounded off to the nearest lowest millimeter mark. PPD was measured at six sites of the tooth: disto-buccal, buccal, mesio-buccal, disto-lingual, lingual and mesio-lingual.

GCF sampling - GCF samples were taken from different sites. Samples were preferably taken from the buccal surfaces of upper premolars and molars. Four GCF sampling sites were identified for each subject. GCF samples were collected using a standard method[26] of periodontal paper strips.[c] First, the quadrant test section was isolated with cotton rolls and the tongue was covered with gauze (if desired). The site was dried with a gentle stream of air; care was taken to ensure that saliva was not present at the sampling site. Periopaper strips were inserted into the pockets until resistance was felt and removed after approximately 30 seconds and placed into the jaws of the calibrated Periotron 8000[c] unit to determine the volume of GCF (reported as microliters) on the strip.[27] The Periotron reading was recorded on the data report form. After the Periotron value was determined, the strip was removed from the jaws using cotton pliers. The orange plastic portion of the strip was cut using ceramic scissors to prevent metal contamination and the absorbent portion was placed into a pre-labeled empty vial. Sites were not sampled more than two times. The Periopaper strip samples were immediately placed on dry ice until transferred to a -80°C freezer.

Analysis on GCF paper strips - Periopaper was prepared for analysis with a mixture of deionized (DI) water and concentrated nitric and hydrochloric acids in a microwave digestion system. The resulting solution was diluted and then analyzed by inductively coupled plasma mass spectrometry [Agilent 8800 ICP-MS/MS[d] (triple quadrupole)] using rhodium (Rh) as an internal standard. Results are reported in ng Sn/µL GCF. Specifically, at the time of sampling, the volume of GCF collected was determined at the clinical site via Periotron measurements on strip samples. For each subject and time point, three blank Periopapers were also collected. The samples were shipped from the clinical site on dry ice to the analysis laboratory and kept in the freezer at -80ºC until they were prepared for analysis. To digest the samples, the Periopaper was transferred to a pre-cleaned 7 mL TFM UltraWAVE vial[e] and combined with 0.25 mL deionized water, 1 mL nitric acid, and 0.2 mL hydrochloric acid. Samples were then digested using an UltraWAVE microwave digestion system,[e] according to the following temperature program: 20 minutes ramp to 250ºC, and 15 minutes hold at 250ºC. Samples were then cooled to below 60ºC and quantitatively transferred to a 15 mL polypropylene tube with the aid of deionized water. After addition of the appropriate internal standard, the solution was diluted to 5 mL with deionized water and mixed well. Prepared standards, test samples and fortified test samples were analyzed using an Agilent 8800 ICP-MS/MS[d] (triple quadrupole), employing detection schemes optimized for each targeted m/z, and appropriate accessories. For each microwave batch, Periopaper was digested and prepared according to the procedural steps outlined above to verify the absence of contamination from the reagents and microwave vials. As an in-process verification of method accuracy and precision, Periopaper was fortified with known levels of tin, and then otherwise prepared per the procedures described above. For each microwave run, a minimum of one spike was prepared, and the spike level alternated between 50 ppt Sn, 500 ppt Sn, and 2,000 ppt Sn (in test solution) between microwave batches. These spike levels are equivalent to 0.25 ng Sn/sample, 2.5 ng Sn/sample, and 10 ng Sn/sample. Working standards were prepared by combining appropriately diluted reference standards,[f] covering the concentration range of interest (20 ppt – 10,000 ppt), together with selected internal standards. The final composition of these working standards was matched to the prepared samples, in terms of acid content, for matrix-matching purposes. Multiple batches of standard solutions were prepared throughout analysis of the samples. Results from ICP-MS/MS analysis of these working standards yielded the requisite data to prepare calibration curves for quantitation of each targeted trace element.

Statistical analysis - Summary statistics (e.g., means, standard deviations, frequencies, etc.) of the demographic characteristics

Table 1. Demographics summary.

Gender	Female	14 (70 %)
	Male	6 (30 %)
Ethnicity	Asian Oriental	2 (10 %)
	Black	1 (5 %)
	Caucasian	16 (80 %)
	Multiracial	1 (5 %)
Age (SD)	41.9 (12.1)	
Gingival Bleeding Index (SD)	0.4 (0.17)	
Gingival Bleeding Sites (SD)	45.4 (15.9)	

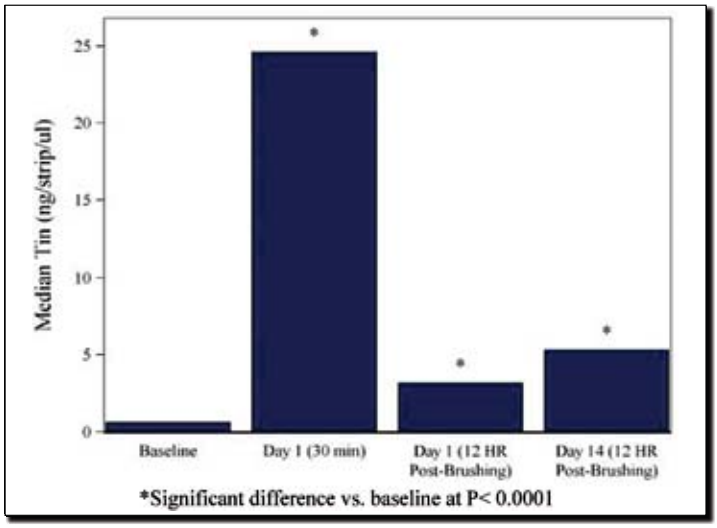

Figure. Tin levels in GCF at each visit.

Table 2. Sn levels in GCF at each visit.

Visit	GCF results of tin levels (ng/μL) n=20 per visit		
	Mean tin (SD)	Median	P value*
Baseline	0.8 (0.9)	0.6	---
30 minutes	33.6 (28.8)	24.6	< 0.0001
Day 1 (12 hours since last brushing)	6.7 (9.3)	3.1	< 0.0001
Day 14 (12 hours since last brushing)	8.1 (7.3)	5.3	< 0.0001

* Wilcoxon signed rank P value comparing visit value vs. baseline.

as well as the efficacy endpoints were calculated for each visit. Statistical analyses were based on average GCF, and values of Sn analyte within a subject. Values of BQL (below quantifiable limit) were set equal to BQL threshold for analysis purposes. A paired t-test was scheduled to be performed to determine the difference between post-baseline visits and baseline. Separate analyses were done for each post-baseline visit (30 minutes, 1 day, and 14 days). If normality assumptions were not satisfied, data transformation was to be performed or non-parametric methods could be alternatively used. For tin levels in GCF, normality assumptions were not met so non-parametric methods (Wilcoxon signed rank test) were used. Statistical tests were two-sided using a 5% significance level.

Results

Table 1 summarizes population statistics for the study including measures of the bleeding index and bleeding sites. Twenty-three subjects attended the screening/acclimation visit with three dropping out; the remaining 20 subjects attended the baseline visit and completed the study protocol. The average number of bleeding sites in this population measured 45 sites.

The Figure and Table 2 summarize subgingival tin concentrations observed in the study at the various visits.

Significant levels of tin were observed in sampled sulci in the analysis of samples from each visit following baseline. Tin determined in the samples ranged from 140 ng Sn/μL GCF to BQL. The highest level of tin was found in the 30-minute post brushing samples. The majority of the study blank and baseline samples were BQL. The median values at the 30-minute, Day 1, and Day 14 collections were 24.59, 3.14, and 5.30ng Sn/μL GCF, respectively. The units of Sn concentration in the GCF can be converted to concentrations of μmol/L by a factor of 8.4, with median values yielding micromolar levels of tin in GCF at clinic visits of 206.6 μmol/L, 26.4 μmol/L and 44.5 μmol/L for 30 minutes, Day 1 12-hour post-brushing and Day 14 12-hour post-brushing samples, respectively.

Discussion

Research in Procter & Gamble laboratories and others have established that toothbrushing with high bioavailable stannous fluoride toothpastes is effective in improving gingival health.[7-16] While the efficacy of stannous fluoride in reducing gingivitis is typically associated with antiplaque efficacy, our laboratory has obtained a more detailed understanding of the antimicrobial mechanisms leading to its gingival health benefits. This research has led us toward the identification of stannous fluoride activity in modification of plaque metabolic production of short chain fatty acids[17] and the chemical deactivation of pathogen endotoxins[18-22] as potential determinants of clinical efficacy. We have chosen to define the metabolic and endotoxin inhibitory properties as pathogen detoxification.

The detoxification of pathogens by stannous fluoride requires direct interaction with bacteria. In clinical studies, we found that subgingival plaque samples taken from subjects brushing with stannous fluoride dentifrice showed reduced reactive endotoxin concentrations measured with dyes and with reporter toll receptor cells.[21,22] Although the findings from these studies supported that stannous fluoride resulted in reductions in subgingival plaque pathogenicity, it was not known if any of these changes were associated with direct reactivity of stannous fluoride in the sulci. In fact it was unknown whether stannous fluoride would even penetrate into the sulci with toothbrushing. Therefore the purpose of the present study was to explore the penetration and delivery of stannous fluoride into sulci following toothbrushing with stannous fluoride toothpaste. Results showed that significant levels of tin could be measured in GCF sampled from sulci following brushing. Levels of tin were highest at 30 minutes following toothbrushing, but were still detected some 12 hours after the last brushing of subjects. μMolar concentrations of stannous fluoride are sufficient to provide antimicrobial and endotoxin deactivation as seen in laboratory studies.[20,28]

While the results of our collection of studies on subgingival effects of stannous fluoride may seem surprising we believe they might have been anticipated. Local antimicrobial therapies, for example, directly target treatment in the gingival sulcus area. In these therapies, antimicrobials or anti-inflammatories are placed for local delivery in subgingival pockets of patients in the form of gels, chips or fibers. These treatments are designed to deliver high concentrations of therapies directly at the site and their effectiveness is well known. It would appear that topical stannous fluoride applied during regular hygiene also has the capability of extending effects into subgingival sites in gingivitis subjects with 2-4 mm pockets. The GCF levels of tin represent levels being cleared from the sulcus following brushing. In paste usage it is expected that stannous fluoride would have the opportunity to repeatedly react with substrates in the sulci including resident bacteria in plaque, calculus and root surfaces potentially building up concentrations over time. In previous pharmaco-kinetic modeling studies,[24] researchers showed an obvious kinetic relationship between saliva and plaque compartments regarding uptake and clearance of stannous fluoride during and following toothbrushing. Stannous fluoride was observed to be cleared from saliva rapidly but was very well retained in gingival plaque. In this study, the slight elevation in tin levels 12 hours post-brushing in the Day 14 samples vs. Day 1 samples may be indicative of the loading of stannous fluoride into subgingival deposits as sites become saturated with stannous fluoride binding. Because the reaction of stannous fluoride with these substrates is essentially irreversible due to the chemical properties of stannous, each topical exposure contributes to binding of endotoxins at these sites.

In conclusion, in this clinical trial, stannous fluoride penetrated 2-4 mm sampling sites and significant levels were retained for 12 hours. The ability of stannous fluoride to penetrate and be retained in the gingival sulcus may contribute to a reduction in plaque virulence and improvements in gingival health.

a. Procter & Gamble, Cincinnati, OH, USA.
b. Hu-Friedy, Chicago, IL, USA.
c. Ora-Flow, New York, NY, USA.
d. Agilent, Santa Clara, CA, USA.
e. Milestone, Inc., Shelton, CT, USA.
f. Inorganic Ventures, Christiansburg, VA, USA.

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