In Vitro and *In Vivo* Evaluations of the Anticalculus Effect of a Novel Stabilized Stannous Fluoride Dentifrice

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Abstract

- **Objective:** To evaluate the effect of a novel stannous fluoride dentifrice with zinc citrate on calculus inhibition using both *in vitro* and clinical models.
- **Methods:** Each investigation tested a novel stabilized 0.454% stannous fluoride dentifrice with zinc citrate as an anticalculus agent (Crest® Pro-Health™ smooth formula) compared to a negative control fluoride dentifrice. The *in vitro* study used the modified Plaque Growth and Mineralization Model (mPGM). Plaque biofilms were prepared and mineralized by alternate immersion of glass rods in human saliva and artificial mineralization solution. Treatments of 25% w/w dentifrice/water slurries were carried out for 60 seconds daily for 6 days, between saliva and mineralization solution immersions. Plaque calcium levels were determined by digestion and inductively coupled plasma optical emission spectroscopy. Student's t-test ($p < 0.05$) was used for statistical analysis. The clinical study was a parallel group, double-blind, randomized, and controlled trial. Following a dental prophylaxis, subjects entered a two-month run-in phase. At the end, they received a Volpe-Manhold Index (V-MI) calculus examination. Eighty (80) qualified subjects who had formed at least 9 mm of calculus on the linguals of the mandibular anterior teeth were re-prophied and randomly assigned to either the stannous fluoride dentifrice or the negative control. Subjects brushed twice daily, unsupervised, during the three-month test period, returning at Weeks 6 and 12 for safety and V-MI examinations. Statistical analyses were via ANCOVA.
- **Results: *In vitro* mPGM**: The stabilized stannous fluoride dentifrice showed 20% less *in vitro* tartar formation, measured as calcium accumulation normalized by biofilm mass, versus the negative control (106.95 versus 133.04 µg Ca/mg biofilm, respectively, $p < 0.05$).
  Clinical Trial: Seventy-eight (78) subjects completed with fully evaluable data. The stannous fluoride dentifrice group had 15.1% less adjusted mean calculus at Week 6 compared to the negative control group ($p = 0.05$) and 21.7% less calculus at Week 12 ($p < 0.01$). Both dentifrices were well-tolerated.
- **Conclusion:** The stannous fluoride dentifrice produced significant anticalculus benefits *in vitro* and in a clinical trial compared to a negative control.

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Introduction

Incomplete daily dental plaque removal, particularly in hard-to-brush areas, is commonplace, and as a result, dental calculus (tartar) is highly prevalent in adults.[1-9] The supragingival and/or subgingival presentation and extent of coverage of dental calculus is variable and influenced by such factors as oral hygiene practices, age, gender, diet, and access to care.[1] Affected individuals are likely to form tartar, at a minimum, in areas of the dentition that are near salivary ducts, such as the mandibular anterior lingual and maxillary molar buccal surfaces (Figure 1).[1] Patients may view tartar as primarily a cosmetic concern, not recognizing the potential increased risk to periodontal health resulting from the propensity of the cement-like supragingival calculus deposits to hinder effective gingival and interproximal cleaning. Because the unsightly, tenacious deposits can only be removed professionally via mechanical scaling, the control of calculus is of considerable value with respect to esthetics, effective oral hygiene, gingival health, and ease of dental prophylaxes.

Unlike dental plaque, the microbial pellicle biofilm, which begins immediately forming again upon a clean tooth surface, dental calculus formation is a slower process, with the potential for prevention via

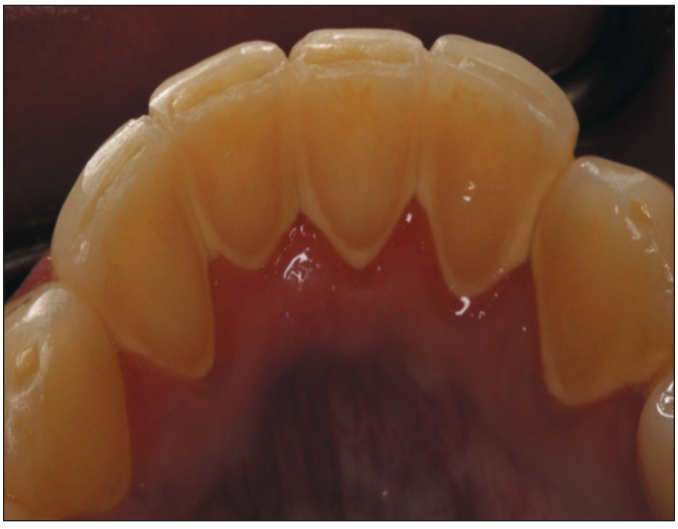


Figure 1. *Supragingival calculus tends to be greater in areas adjacent to the salivary glands, such as on the mandibular anterior lingual tooth surfaces.*

thorough oral hygiene or clinically efficacious antitartar agents.[10,11] Supragingival dental plaque biofilms, left undisturbed to mature via insufficient tooth brushing/interdental cleaning, can ultimately mineralize and calcify, becoming too hard for self-removal by the individual.[1] This process is initiated when plaque absorbs salivary calcium and phosphate, proceeding more rapidly in areas adjacent to the salivary ducts.[1,12-14] Crystallization phases follow at a pace mitigated by endogenous and exogenous factors (*e.g.*, salivary ion levels and dietary components), with the calcium mineral phosphate salts interspersed in the matrix between organic and inorganic microorganisms.[1,12-14] The resulting crystalline aggregates vary in structure and composition impacted by mineral nucleation and the age of the deposits.[1] Friskopp, *et al.* conducted a microradiographic study revealing that supragingival calculus was seemingly heterogeneous and stratified with some areas appearing to be non-calcified.[15] A mature, petrified calculus serves as a porous substratum for bacterial plaque, with an outer plaque layer of predominately gram-negative microorgansims.[16,17]

For several decades, the key supragingival calculus-fighting strategy has been the attempt to inhibit and slow the mineralization/crystallization of plaque with the topical use of chemotherapeutic products, thus reducing the extent of tartar accumulation and allowing a longer window of time for soft, non-mineralized deposits to be removed through routine mechanical oral hygiene. Many commercially available toothpastes and mouthrinses make tartar control claims and contain an anticalculus ingredient, typically pyrophosphate, sodium hexametaphosphate, Gantrez copolymer, or zinc salts.[18-22]

Crest® Pro-Health® dentifrice (Procter & Gamble Company, Cincinnati, OH, USA) with stabilized 0.454% stannous fluoride and sodium hexametaphosphate, introduced in 2005, was the first dentifrice to simultaneously provide the therapeutic benefits of stannous fluoride with stain inhibition and calculus control.[21,22] Recently, a smooth texture formulation of Crest Pro-Health, containing zinc citrate as the tartar control agent in place of sodium hexametaphosphate, was introduced, offering patients the same benefits but with a unique texture, cleaning experience, and flavors. Both an *in vitro* investigation and a randomized and controlled clinical study were executed to evaluate the calculus inhibition efficacy of the novel smooth texture dentifrice relative to non-tartar control, fluoride dentifrice.

Materials and Methods

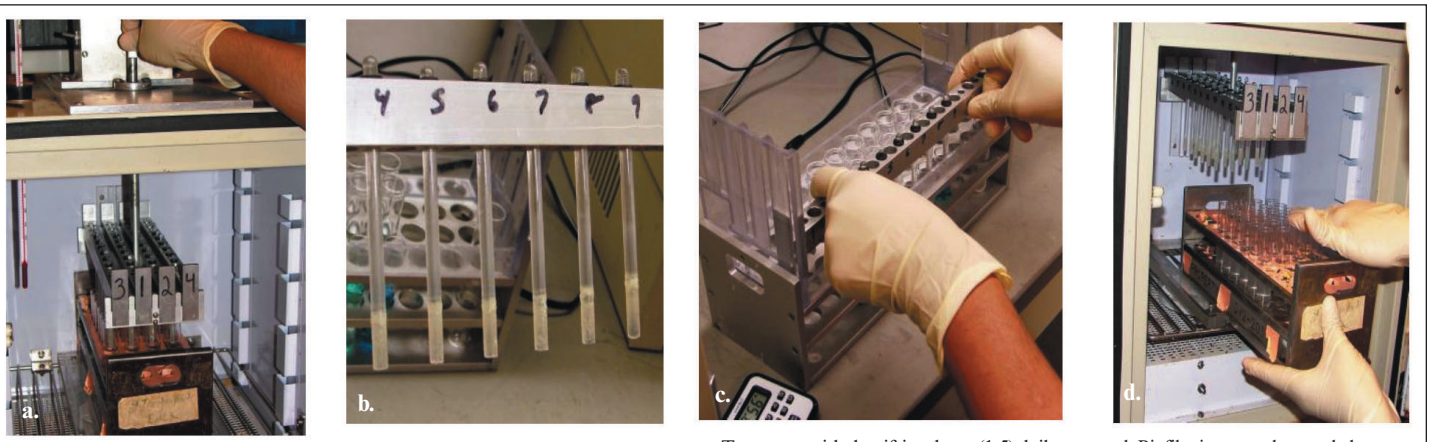
In Vitro *Investigation*

One means of predicting the tartar control performance of dentifrices *in vivo* is via the use of the *in vitro* modified Plaque Growth and Mineralization Model (mPGM), an established, validated plaque biofilm calcification model.[23] With this method, the respective calculus inhibition efficacy of the novel stannous fluoride dentifrice, Crest Pro-Health smooth texture dentifrice, and a negative control sodium fluoride dentifrice (Crest® Cavity Protection, Procter & Gamble Company, Cincinnati, OH, USA) were evaluated.

Plaque biofilm growth was initiated by dipping polished glass rods overnight at 37°C into a medium of fresh pooled human saliva (60% v/v) and trypticase soy broth (TSB, 40% v/v). For the establishment of biofilm on the rods, the medium was exchanged on the morning of the second day to a sucrose-rich broth. Biofilm was grown with growth medium (TSB 15 g), sucrose (50 g), and deionized water (467 ml), supplemented with freshly pooled saliva (33 g). The medium was changed again after five hours and biofilm was grown overnight in supplemental pooled saliva (10% v/v TSB) and 1.25% w/v sucrose. The two-day biofilms were treated with the 25% dentifrice/water slurries (1:5) for 60 seconds, then rinsed by immersing each glass rod twice for 10 seconds into deionized water. The treated rods were then exposed to a calcium-containing mineralization solution for at least four hours, rinsed by dipping each glass rod twice for 10 seconds into deionized water, and finally exposed to human saliva overnight. This entire sequence of treatment/mineralization/biofilm growth was conducted once daily for six days (Figure 2). Following this six-day cycling of treatment, the plaque biofilm was removed from the rods and digested using potassium hydroxide, hydrochloric acid, and acetic acid. The samples were vortexed and the rods were removed from solution. The respective plaque calcification levels were then determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), with Student's t-test ($p < 0.05$) used for statistical analysis.

Clinical Trial

A randomized, double-blind, parallel group, single-center clinical study was conducted in two phases: a two-month run-in phase and a three-month treatment phase, with generally healthy adult subjects (Figure 3). The protocol and subject consent form were approved by



a. Glass rods dip into saliva/TSB at 37°C.

b. Two-day biofilm grown from human saliva.

c. Treatment with dentifrice slurry (1:5) daily for six days.

d. Biofilm is exposed to pooled human saliva/mineralization solution.

Figure 2. *The key steps in the modified Plaque Growth and Mineralization (mPGM) method for analysis of* in vitro *plaque biofilm mineralization.*

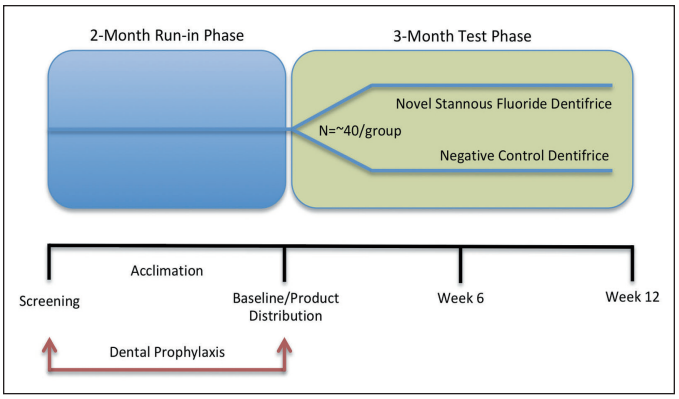


Figure 3. *The clinical trial design incorporated a two-month run-phase, followed by a three-month test phase.*

the U.S. Institutional Review Board (U.S.IRB2013SRI/03) before study initiation, and verbal and written consent were obtained from all subjects. For inclusion, all volunteers needed a minimum of 16 natural teeth, including six mandibular anterior teeth with no crowns or veneers. Any subject who had a medical condition requiring antibiotic premedication prior to dental procedures, was a regular user of a chlorhexidine mouthrinse, or had any oral conditions or pathoses that could interfere with study compliance and/or examination procedures (*e.g.*, widespread caries, chronic neglect, soft or hard tissue tumors, advanced periodontal disease) was not eligible for study enrollment. In addition, during the course of the trial, subjects who used non-study oral hygiene products, did not comply with product usage instructions, or who received elective dentistry or a dental prophylaxis could be excluded from the data analyses or withdrawn from the study.

At the inception of the two-month run-in/screening phase to evaluate supragingival calculus formation, participants meeting all entrance criteria received an oral soft tissue examination and a Volpe-Manhold Index (V-MI) calculus examination[24] on the lingual surfaces of the six mandibular anterior teeth by an experienced clinical examiner. They then received a complete dental prophylaxis. Subjects were provided with regular, marketed Colgate® Cavity Protection toothpaste (Colgate-Palmolive, New York, NY, USA) and an American Dental Association (ADA) reference soft manual toothbrush (Chicago, IL, USA) and instructed to brush at home twice daily (morning and evening) with a full brush head of toothpaste for one minute for the duration of the screening phase.

At the end of this two-month run-in phase, subjects were recalled for V-MI examinations to determine eligibility for continuation in the subsequent test phase of the clinical trial. Those who had demonstrated a propensity for calculus formation as evidenced by at least 9 mm of calculus on the lingual surfaces of the six mandibular teeth, and who continued to meet all other study entrance criteria, were qualified to continue participation. At this baseline visit for the second phase of the trial, the continuing subjects were evaluated for oral soft tissue health, and provided with a complete prophylaxis to return supragingival calculus scores to zero. Subjects were stratified by baseline lingual V-MI calculus scores, gender, and age. Outside of the presence of the clinical examiner for maintenance of blinding, they were then randomly assigned, using a computer-encoded program, to the stannous fluoride dentifrice group or the negative control dentifrice group.

As in the run-in/screening phase, subjects were directed via both oral and written instructions to brush twice daily for one minute with their assigned dentifrice using the supplied ADA reference soft manual toothbrush. Although all product usage was at home during the three-month test phase, an initial brushing at the clinical site under staff supervision was conducted to verify understanding of the product use instructions. All dentifrices were overtubed/overlabeled/overwrapped to preclude identification, and supplied in identically appearing test kits along with the toothbrush and timer for blinding assurance.

At Week 6 and Week 12 of the test phase, subjects presented for safety evaluations and V-MI calculus efficacy assessments to determine the relative effects of twice-daily home use of the two dentifrices, following confirmation of continued study eligibility. For safety, a thorough evaluation of the oral soft tissues was conducted by way of a visual examination of the oral cavity, including 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.

To assess clinical efficacy, the V-MI quantified supragingival calculus present on the lingual surfaces of six mandibular anterior teeth.[24] After drying the teeth with a stream of air and using a standard periodontal probe graduated in millimeters, the examiner placed the instrument on the most inferior border of the visible calculus, and measurements were obtained on the following three planes:

1) bisecting the center of the lingual surface;
2) diagonally through the mesial-incisal point angle of the tooth through the area of greatest calculus height; and
3) diagonally through the distal point angle of the tooth through the area of the greatest calculus height.

The examiner assigned a score to each measurement plane, with measurements made in 0.5 mm increments starting at 0.5. A score of zero (0) denoted that there was no calculus present at a measurable site. The V-MI was calculated for each subject by summing the millimeter scores over all sites graded.

Adverse event reports were summarized by test group. Summary statistics (*e.g.*, means, standard deviations, frequencies) of the baseline demographic characteristics and the V-MI efficacy measurements were calculated for each dentifrice test group and study visit. Test groups were compared using the analysis of covariance (ANCOVA) method; all statistical tests were two-sided with a 5% level of significance. The anticalculus efficacy response was the V-MI score at Week 6 and Week 12, and the covariate was the Phase 2 baseline V-MI score. Due to lack of normality of the data at Week 6, an outlier test was performed. Based on the Dixon's test for statistical outliers,[25] a subject in the negative control dentifrice group was determined to be an outlier, and data was excluded from the analysis at Week 6.

Results

In Vitro *Investigation*

Results of the mPGM investigation are shown in Table I. The stabilized stannous fluoride dentifrice showed 20% less *in vitro* plaque biofilm calcification relative to the negative control dentifrice. Calcium accumulation normalized by biofilm mass for the stannous fluoride and control dentifrices was 106.95 µg/mg and 133.04 µg/mg, respectively ($p < 0.05$).

Table I
In Vitro Plaque Mineralization Inhibition Results (mPGM)

	Calcium µg/mL (SD)	Calcium/Biofilm Mass µg/mL (SD)	% Inhibition Versus Comparator	p-value[a]
Stannous Fluoride	19.85 (4.40)	106.95 (20.79)	20%	< 0.05
Negative Control	28.56 (3.27)	133.04 (14.93)		

mPGM = modified Plaque Growth and Mineralization method;
SD = standard deviation;
% = percentage
[a]Based on Student's t-test ($p < 0.05$)

Clinical Trial

A total of 92 subjects provided informed consent and were enrolled during the Phase I run-in/screening phase, and 80 of these met the Phase 2 test phase entrance criteria and were randomized at baseline to either the stannous fluoride or negative control dentifrice. Two subjects in the negative control group discontinued study participation prior to study end, with 78 subjects (98%) completing and deemed fully evaluable at the trial's conclusion. As shown in Table II, the mean age of the randomized study population was 52 years, with a range of 19 to 80 years; forty-six (58%) of the subjects were female. The test phase study population was well-balanced with respect to all baseline demographic variables ($p \geq 0.2998$).

At baseline before prophylaxis, the test groups did not differ statistically significantly in mean V-MI calculus levels ($p = 0.3542$), where the stannous fluoride group's average score was 17.56 (range 9.00–43.00) and the mean control group V-MI score was 18.99 (range 9.50–45.50; Table II).

Table III and Figure 4 summarize the calculus-inhibiting efficacy results from the three-month test phase. At Week 6, the adjusted mean V-MI score was 12.80 for stannous fluoride, compared with 15.08 for the negative control. The V-MI score between-group difference of 2.28, numerically favoring the stannous fluoride dentifrice, represented a 15.1% lower calculus score versus the negative control ($p = 0.0521$).

Table II
Baseline Subject Characteristics – Randomized Subjects

	Stannous Fluoride Dentifrice	Negative Control	Overall
Characteristic	n = 41	n = 39	n = 80
Mean Age (SD)[a]	51.2 (12.38)	52.7 (12.13)	52.0 (12.20)
Age Range	23–80	19–80	19–80
Female (n, %)[b]	23 (56%)	23 (59%)	46 (58%)
Male (n, %)[b]	18 (44%)	16 (41%)	34 (43%)
Asian Oriental[b]	1 (2%)	0 (0%)	1 (1%)
Black[b]	5 (12%)	2 (5%)	7 (9%)
Caucasian[b]	34 (83%)	37 (95%)	71 (89%)
Hispanic[b]	1 (2%)	0 (0%)	1 (1%)
V-MI mean (SD)[c]	17.56 (6.23)	18.99 (7.43)	18.26 (6.84)
V-MI Min.-Max.	9.00–43.00	9.50–45.50	9.00–45.50

n = number of subjects; SD = standard deviation; V-MI = Volpe Manhold Calculus Index; Min.-Max. = Minimum – Maximum Mean Score
[a]Two-sided ANOVA for the between-group mean age comparison ($p = 0.5739$).
[b]Two-sided Fisher's Exact Test for the between-group gender balance comparison ($p = 0.8244$) and for the between-group ethnicity balance comparison ($p = 0.2998$).
[c]Two-sided ANOVA for the between-group mean V-MI calculus comparison ($p = 0.3542$).

At Week 12, the difference between the two dentifrices was more pronounced, with a 21.7% lower calculus score for the stannous fluoride group compared to the negative control group ($p = 0.006$). Mean V-MI Week 12 scores were 13.28 and 16.95 for the stannous fluoride and negative control groups, respectively, with a between-group difference favoring stannous fluoride of 3.67. Both dentifrices were well-tolerated; no adverse events were reported.

Table III
ANCOVA Volpe-Manhold Index Calculus Treatment Comparisons: Week 6 and Week 12 Results

	Adjusted Mean (SE)	Treatment Difference (SE)	% Difference Versus Negative Control[a]	Two-sided p-value
Week 6				
Stannous Fluoride (n = 37)	12.800 (0.795)	2.276 (1.153)	15.1%	0.0521
Negative Control (n = 41)	15.076 (0.836)			
Week 12				
Stannous Fluoride (n = 37)	13.275 (0.894)	3.672 (1.298)	21.7%	0.0061
Negative Control (n = 41)	16.947 (0.941)			

SE = standard error; % = percentage; n = number of subjects
[a]Percent change versus negative control = 100 X (Negative Control – Stannous Fluoride/Negative Control)

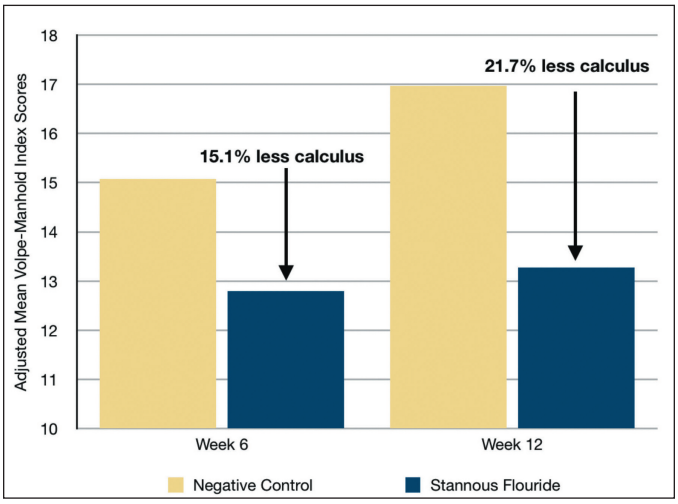


Figure 4. *The stannous fluoride dentifrice provided a calculus inhibition benefit compared to the negative control toothpaste at both Weeks 6 ($p = 0.052$) and 12 ($p = 0.006$).*

Discussion

Even in populations who practice oral hygiene and have access to regular professional care, it is estimated that between 50% and 100% of adults have at least some supragingival calculus formation.[26] With interest in teeth whitening and an attractive smile being most popular historically, the chalky, yellowish-appearing deposits that are prone to attract and acquire stains through diet and/or habits can be noticeable on facial surfaces and cosmetically undesirable.

Patients who find their tartar build-up objectionable do not have a self-care option for removal; the deposits obtain a remarkable hardness and tenacity once fully mineralized[1,27] that can only be addressed with professional dental scaling. Heavier accumulations may necessitate longer, more frequent, and/or more uncomfortable

scaling sessions, with the potential for greater expenditures of finances, as well as time (both patient and clinician) and professional effort. Avoiding or delaying dental evaluations and prophylaxis appointments for any of these reasons comes with obvious implications for the patient's oral health.

In contrast to the inconveniences inherent with removal, preventing or reducing the extent of calculus before it is established is achievable, and provides the motivating prospect to patients of easier, more pleasant dental cleanings. Dentifrices with clinically proven anticalculus agents are an easy-to-implement means of reducing tartar, and both consumers and clinicians benefit from research to aid in selecting the best products. Reproducible laboratory testing can aid manufacturers in screening formulations and predicting the outcome of subsequent clinical testing. The modified Plaque Growth and Mineralization test utilized in the *in vitro* investigation herein is one such method for projecting the outcome of clinical product comparisons. In finding the novel stannous fluoride dentifrice to yield 20% less plaque mineralization versus the control, mPGM proved to be highly predictive of the *in vivo* outcome.

In the present 12-week clinical trial test phase, the calculus inhibition effects of the novel stabilized 0.454% stannous fluoride dentifrice with zinc citrate were compared to those of a negative control. Zinc salts have been, and continue to be, successfully used in marketed anticalculus products based on their documented ability to reduce plaque growth and disrupt and slow crystal formation; specifically, positively charged zinc ion (Zn^{2+}) inhibits crystal growth by substituting for calcium in the crystal lattice of calcium phosphate.[18,28,29] Zinc citrate is a widely recognized anticalculus agent, and replaced zinc chloride in tartar control dentifrices because citrate provides the added benefit of crystal aggregation inhibition and does not have an unpleasant taste.[4] Clinical trials dating back to 1987, with diverse study designs and differing controls, have demonstrated statistically significant superior tartar control benefits for zinc citrate in various dentifrice formulations.[1,18,30] Zinc citrate has also been shown to exhibit good oral retention in saliva and plaque following tooth brushing.[29,31] The results of this trial, where the stannous fluoride dentifrice provided up to 22% greater calculus inhibition versus a control with increasingly greater relative benefits with longer use, confirmed the chemotherapeutic ability of an anticalculus dentifrice with zinc citrate to effect significant tartar control. The study was well-controlled, with a lengthy screening phase to ensure subjects were natural calculus formers (and therefore would be representative of intended users), unsupervised home use consistent with real-world usage, and blinded products to prevent bias.

Patients increasingly seek not only effective products for their cosmetic and therapeutic needs, but products that can offer multiple benefits in one source for added simplicity and value. Tartar control is seldom the only oral health need, so a dentifrice that supplies this benefit, and is also effective for numerous other needs/wants, is ideal. The novel dentifrice in these investigations designed for enhanced esthetics and consumer acceptability is a multi-indication product with the broad benefits uniquely afforded by stabilized stannous fluoride, that can provide not only highly effective caries and calculus protection, but also significant control or reduction of plaque, gingivitis, halitosis, dentinal hypersensitivity, and enamel erosion.[32-36] Additionally, silica provides stain removal and whitening[37] in this new dentifrice targeting an extensive range of oral health diseases and conditions.

Conclusion

The stabilized stannous fluoride dentifrice with zinc citrate produced significant anticalculus benefits *in vitro* and in a clinical trial compared to a negative control. These results demonstrate that the mPGM measure is a meaningful parameter to forecast *in vivo* calculus formation.

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Conflict of Interest: Dr. He, Ms. Anastasia, Dr. Schneiderman, Dr. Zsiska, and Ms. Farmer are employees of Procter & Gamble. Dr. Milleman has no conflicts to disclose.

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References

1. White DJ. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *Eur J Oral Sci* 1997;105:508-22.
2. Beiswanger BB, Segreto VA, Mallatt ME, Pfeiffer HJ. The prevalence and incidence of dental calculus in adults. *J Clin Dent* 1989;1:55-8.
3. Albandar JM, Kingman A. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 1999;70:30-43.
4. Mandel ID. Calculus update: prevalence, pathogenicity and prevention. *J Am Dent Assoc* 1995;126:573-80.
5. Gökalp SG, Doan BG, Tekçiçek MT, Berbero lu A, Unlüer S. National survey of oral health status of children and adults in Turkey. *Community Dent Health* 2010;27:12-7.
6. Coelho Rde S, Gusmão ES, Jovino-Silveira RC, Caldas Ade F. Profile of periodontal conditions in a Brazilian adult population. *Oral Health Prev Dent* 2008:6:139-45.
7. Corbet EF. Periodontal diseases in Asians. *J Int Acad Periodontol* 2006;8:136-44.
8. Morris AJ, Steele J, White DA. The oral cleanliness and periodontal health of UK adults in 1998. *Br Dent J* 2001;191:186-92.
9. McFall WT Jr, Bader JD, Rozier RG, Ramsey D, Graves R, Sams D, Sloame B. Clinical periodontal status of regularly attending patients in general dental practices. *J Periodontol* 1989;60:145-50.
10. Bollmer BW, Sturzenberger OP, Vick V, Grossman E. Reduction of calculus and Peridex stain with Tartar-Control Crest. *J Clin Dent* 1995;6:185-7.
11. Al-Hashimi I, Levine MJ. Characterization of *in vivo* salivary-derived enamel pellicle. *Arch Oral Biol* 1989;34:289-95.
12. Mandel ID. Calculus formation and prevention: an overview. *Compend Contin Educ Dent* 1987;8(Suppl):S235-41.
13. Nancollas GH, Johnsson MA. Calculus formation and inhibition. *Adv Dent Res* 1994;8:307-11.
14. Mandel ID. Biochemical aspects of calculus formation. II. Comparative studies of saliva in heavy and light calculus formers. *J Periodontal Res* 1974;9:211-21.
15. Friskopp J, Isacsson G. A quantitative microradiographic study of mineral content of supragingival and subgingival dental calculus. *Scand J Dent Res* 1984;92:25-32.
16. White DJ. Tartar control dentifrices: current status and future prospects. In: Embery, Rolla, eds. *Clinical and Biological Aspects of Dentifrices*. Oxford: Oxford University Press; pp. 277-91, 1992.
17. Sidaway DA. A microbiological study dental calculus. II. The *in vitro* calcification of microorganisms from dental calculus. *J Periodontal Res* 1978;13:360-6.
18. Van Loveren C, Duckworth RM. Anti-calculus and whitening toothpastes. *Monogr Oral Sci* 2013;23:61-74.
19. White DJ, Gerlach RW. Anti-calculus effects of a novel, dual-phase polypyrophosphate dentifrice: chemical basis, mechanism, and clinical response. *J Contemp Dent Pract* 2000;1:1-19.
20. White DJ, McClanahan SF, Lanzalaco AC, Cox ER, Bacca L, Perlich MA, Campbell SL, Schiff T, Stains A. The comparative efficacy of two commercial tartar control dentifrices in preventing calculus development and facilitating easier dental cleanings. *J Clin Dent* 1996;7(2 Spec No):58-64.
21. Schiff T, Saletta L, Baker RA, He T, Winston JL. Anti-calculus efficacy and safety of a stabilized stannous fluoride/sodium hexametaphosphate dentifrice.

Compend Contin Educ Dent 2005;26(9 Suppl 1):29-34.
22. Winston JL, Fiedler SK, Schiff T, Baker R. An anti-calculus dentifrice with sodium hexametaphosphate and stannous fluoride: a six-month study of efficacy. *J Contemp Dent Pract* 2007;8:1-8.
23. White DJ, Cox ER. *In vitro* studies of the anticalculus efficacy of a sodium hexametaphosphate whitening dentifrice. *J Clin Dent* 2002; 13:33-37.
24. Volpe AR, Manhold JH, Hazen SP. *In vivo* calculus assessment: Part 1. A method and its examiner reproducibility. *J Periodontol* 1965; 36: 292-8.
25. Barnett V, Lewis T. *Outliers in Statistical Data*. New Jersey:John Wiley & Sons, pp. 91, 97-98, 311, 1978.
26. Anerud A, Löe H, Boysen H. The natural history and clinical course of calculus formation in man. *J Clin Periodontol* 1991;18:160-70.
27. White DJ. Processes contributing to the formation of dental calculus. *Biofouling* 1991;4:209-18.
28. Saxton CA, Harrap GJ, Lloyd AM. The effect of dentifrices containing zinc citrate on plaque growth and oral zinc levels. *J Clin Periodontol* 1986;13: 301-6.
29. LeGeros RZ, Bleiwas CB, Retino M, Rohanizadeh R, LeGeros JP. Zinc effect on the in vitro formation of calcium phosphates: relevance to clinical inhibition of calculus formation. *Am J Dent* 1999;12:65-71.
30. Fairbrother KJ, Heasman PA. Anti-calculus agents. *J Clin Periodontol* 2000:27:285-301.
31. Gilbert RJ, Ingram GS. The oral disposition of zinc following the use of an anticalculus toothpaste containing 0.5% zinc citrate. *J Pharm Pharmacol* 1988;40:399-402.
32. Zhao X, He T, He Y, Cheng C, Chen H. A randomized clinical trial to measure the erosion protection benefits of a novel stabilized stannous fluoride dentifrice versus a control dentifrice. *J Clin Dent* 2017;28(Spec Iss B):B17-20.
33. He T, Anastasia MK, Eusebio R, Whalen SD. The oral malodor reduction efficacy of a 0.454% SnF_2 dentifrice. *J Dent Res* 2017;96(Spec Iss A):Abs 890.
34. Friesen L, Goyal CR, Qaqish, He T, Eusebio R, Zsiska M, Farmer T, Schneiderman E. Comparative anti-plaque effect of two antimicrobial dentifrices: laboratory and clinical evaluations. *J Clin Dent* 2017;28(Spec Iss B): B6-11.
35. He T, Eusebio R, Goyal CR, Qaqish JG. Assessment of the effects of a novel stabilized stannous fluoride dentifrice on gingivitis in a two-month positive-controlled clinical study. *J Clin Dent* 2017;28(Spec Iss B):B12-16.
36. He T, Farrell S. The case for stabilized stannous fluoride dentifrice: an advanced formulation designed for patient preference. *J Clin Dent* 2017;28(Spec Iss B) B1-5.
37. Friesen L, He T. Eusebio R. Extrinsic stain removal efficacy of a 0.454% stannous fluoride dentifrice. *J Dent Res* 2017;96(Spec Iss A):Abs 1941.