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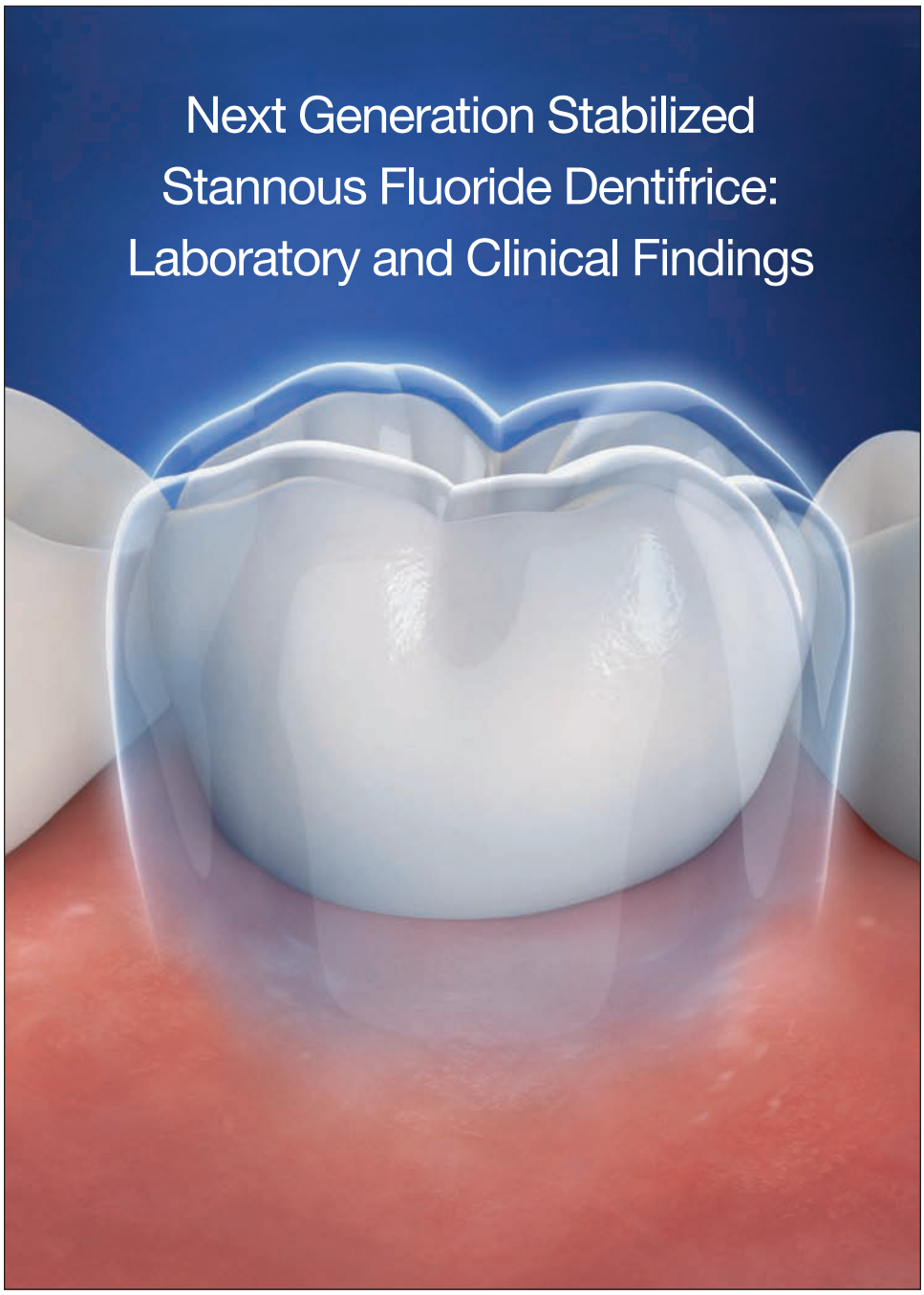

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On the Cover
Cover art depicts protection to hard and soft tissues from use of stabilized stannous fluoride dentifrice.

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The Case for Stabilized Stannous Fluoride Dentifrice: An Advanced Formulation Designed for Patient Preference

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Abstract

Oral diseases, particularly caries and gingivitis, continue to be widespread. Incorporating a stabilized stannous fluoride dentifrice into patients' daily oral hygiene routine is a convenient, cost effective approach to improve and protect their oral health and the appearance of their smile. Unlike other common fluorides that only provide anti-caries benefits (*e.g.*, sodium fluoride and sodium monofluorophosphate), stabilized stannous fluoride formulations have demonstrated broader and significantly greater protection, also reducing plaque, gingivitis, erosion, sensitivity, and halitosis. To deliver the full range of benefits and simultaneously deliver whitening and tartar control benefits, stannous fluoride requires careful formulation. Procter & Gamble is the only dentifrice manufacturer with decades of patented innovations to overcome these formulation challenges, resulting in a large portfolio of stannous fluoride-containing dentifrice products marketed under the Crest® Pro-Health™ name that are available today. The most recent innovation is a "smooth texture" variant of Crest Pro-Health, containing stabilized stannous fluoride with zinc citrate as the anti-calculus agent. This product was developed to deliver a patient preferred brushing experience with the full range of benefits offered by Crest Pro-Health. This article discusses two common misconceptions about dentifrice, describes the history of key Crest stannous fluoride innovations, and outlines the research in this issue demonstrating health and cosmetic benefits of the new Crest Pro-Health smooth texture variant.

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Introduction

Oral diseases continue to be pervasive globally.[1] Despite strides in heightened public awareness of the etiology and treatment strategies for conditions such as dental caries and gingivitis, the prevalence of oral diseases and conditions continues to be high, contributing to both diminished quality of life and growing societal healthcare expenditures.[1] The global burden of oral conditions increased from 1990-2015, mainly due to population growth and aging.[2] In 2015, untreated oral conditions affected 3.5 billion people – approximately 50% of the global population – which was an increase of 40% compared to 1990. Untreated caries in permanent teeth was the most prevalent condition.[2] The Centers for Disease Control and Prevention reports that about one-half of Americans have periodontal disease, with a prevalence of 70% among adults aged 65 years and older.[3]

Two of the most prevalent oral diseases – dental caries and periodontal disease – have multi-factorial determinants but share a common origin, *i.e.*, dental plaque biofilm.[4,5] Plaque deposits left behind from inadequate self-care proliferate and produce caries-promoting acids and a pathogenic microbiota that irritates and inflames the gingiva.[6,7] Targeting plaque is thus critical to disease prevention. Patients need counsel on beneficial interventions to lower their risk of both conditions. Dental professionals have an opportunity to educate patients about the benefits of incorporating clinically proven chemotherapeutic products such as stannous fluoride dentifrice into their oral hygiene routines, but it is often hindered by two frequent misconceptions.

Common Misconceptions About Toothbrushing and Fluoride Dentifrices

Misconception #1: The Dentifrice Doesn't Really Matter Because Mechanical Oral Hygiene is Most Important.

Manual tooth brushing is the most utilized means of oral hygiene. Mechanical plaque removal has the potential to thoroughly remove supragingival plaque, a key causative agent of caries and gingivitis.[8] This can only be realized, however, when mechanical oral hygiene is performed routinely and with sufficient skill and adequate time to access and debride all regions of the dentition. How realistic is this? Research related to the feasibility of attaining that goal is not encouraging, showing that most do not follow the generally recommended two-minute/twice-daily tooth brushing regimen, and plaque commonly remains on tooth surfaces, at the gumline, and in interproximal surfaces. Research on oral hygiene practices has shown:

- Tooth brushing with a conventional manual toothbrush as typically practiced does not remove all plaque. A large systematic clinical trial review by Slot, *et al.* reported that the mean plaque score decrease following a single manual brushing session was about 42%.[9]
- Brushing sessions are not long enough, and individuals overestimate the length of their tooth brushing times. In a series of controlled exercises by Saxer and colleagues, subjects estimated their brushing times at 134–148 seconds, when in reality the actual means were 73–84 seconds.[10] Dentino, *et al.* found that only 17% of uninstructed manual toothbrush users brushed at least two minutes; 66% of the power toothbrush users did so.[11] Beals, *et al.* studied 173 adults in a home-use evaluation and noted an average brushing time of 46 seconds.[12]
- Patients do not adhere to recommended brushing techniques. Several manual tooth brushing techniques (*e.g.*, Bass) have been proven effective and are taught in clinical settings, but an estimated 90% of individuals likely revert to their own customary method of brushing ("scrub method") regardless of its utility.[13]

Retained plaque can lead to calculus deposits that are cosmetically undesirable. Dental plaque and its pathogens also play an important role in the initiation and progression of periodontal disease. When

unaddressed, periodontal disease has the potential to result in bone and tooth loss, and potentially systemic implications.[14-16]

If manual toothbrushing skill is typically inadequate and interdental cleaning is lacking, relying on mechanical oral hygiene to provide a singular defense against oral disease is not realistic. However, nearly all persons utilize toothpaste when brushing, and a dentifrice provides an excellent vehicle for the incorporation of ingredients, such as fluoride for caries protection and chemotherapeutic agents that target gingival disease. Selecting a highly efficacious and clinically proven dentifrice is paramount.

Misconception #2: All Fluoride Dentifrices are the Same.

Fluoride dentifrice has been a standard caries-fighting preventive strategy since Crest® toothpaste with stannous fluoride received the first American Dental Association Seal of Acceptance in 1960. The fluorides most commonly used in commercially available dentifrices today are sodium fluoride (NaF), sodium monofluorophosphate (MFP), and stannous fluoride (SnF_2). Each of these fluorides provides cariostatic benefits via protection against demineralization and promotion of remineralization. As hydroxyapatite crystal dissolution occurs (demineralization) due to pH fluctuations in the tooth surface biofilm, fluoride enhances remineralization by serving as a mineral growth catalyst and by providing lower enamel solubility to protect and prevent it from dissolving, therefore impeding the growth of a carious lesion.[17-19]

While NaF-, MFP-, and SnF_2-containing dentifrices have all been shown to provide caries protection in clinical trials,[20] long-term clinical research has demonstrated greater anti-caries efficacy for stannous fluoride over sodium fluoride.[21,22] Stannous fluoride, when formulated with high bioavailability, is unique among these three fluoride compounds because it offers comprehensive benefits against plaque, gingivitis, enamel erosion, dentinal hypersensitivity, and halitosis[23-33] (Figure 1).

Fluoride Type	Sodium Fluoride	Sodium Monofluorophosphate	Stannous Fluoride*
Plaque			✓
Gingivitis			✓
Erosion			✓
Sensitivity			✓
Halitosis			✓

*All three fluoride compounds provide anti-caries benefits, but stabilized stannous fluoride has demonstrated broader and significantly greater protection for these 5 conditions.

Figure 1. *Stannous fluoride offers broader and greater protection relative to other fluorides.*

Each of these five benefits above and beyond caries protection has been substantiated in rigorous laboratory and clinical testing in independent investigations across a wide range of subject populations and study designs. Sharma, *et al.*,[23] Garcia-Godoy and colleagues,[24] and He, *et al.*[25] have demonstrated stabilized stannous fluoride's significant anti-plaque benefits relative to various controls (including triclosan/copolymer) in short- and longer-term trials utilizing classic examiner-graded plaque indices or digital imaging. Anti-plaque benefits would be expected to confer anti-gingivitis benefits, and indeed research by Mallatt, *et al.*,[26] Archila, *et al.*,[27] and Mankodi and colleagues,[28] among others, has documented the ability of a stabilized stannous fluoride dentifrice to yield significantly greater control of gingival bleeding and inflammation compared to triclosan/copolymer and other control dentifrices.

Enamel erosion is a concern for many, including adolescents consuming high-sugar, acidic popular beverages. Stabilized stannous fluoride dentifrices provided significantly greater erosion protection relative to NaF or MFP/arginine dentifrices in both *in vitro* and *in situ* research published by Hooper, *et al.* and West, *et al*, respectively.[29,30] Likewise, with the commonly experienced condition of dentinal hypersensitivity, stabilized stannous fluoride dentifrice significantly outperformed controls containing other fluoride compounds in reducing sensitivity in clinical trials by Schiff and colleagues (versus NaF) and He, *et al.* (versus SMFP), respectively.[31,32] Stabilized stannous fluoride dentifrice also provided clinical benefits against oral malodor (halitosis) that were significantly greater than that of a sodium fluoride control, as shown in two independent clinical investigations by Farrell and colleagues.[33]

Innovations in Stabilized Stannous Fluoride Formulation

Given the multiple indications that a stannous fluoride dentifrice can simultaneously address, it would seem logical to incorporate this fluoride in more commercially available toothpastes. However, due to the unique properties of stannous fluoride, formulation expertise and skill are a prerequisite to ensure it is delivered in a bioavailable, stable, and esthetically pleasing dentifrice. Recognizing that stannous fluoride provides the broadest protection for patients, scientists at Procter & Gamble have developed numerous patented formulations during the past five decades that overcome the formulation challenges.

In the mid-1950s, the original Crest formulation with stannous fluoride (trademarked as Fluoristan™; Figure 2) had undisputed clinical anti-caries efficacy (up to 53%),[34] however the stannous fluoride

Figure 2. *Original Crest with Fluoristan.*

had low stability and bioavailability, limiting its other therapeutic benefits. Continued research efforts within the Procter & Gamble scientific community led to a series of technical breakthroughs that resulted in the re-emergence of stannous fluoride. In the 1990s, stabilized, bioavailable stannous fluoride dentifrice was successfully formulated due to a new abrasive system and chelant system that increased the chemical stability of the dentifrice (Crest® Gum Care). This formulation was shown in numerous clinical trials to provide a high level of plaque and gingivitis control, though there were esthetic issues (*e.g.*, astringent taste, extrinsic staining potential).[35,36] This led to the next formulation challenge, *i.e.*, delivering the therapeutic benefits of stannous fluoride

while concurrently providing cosmetic benefits like tooth whitening. Ten years later, a novel stabilized stannous fluoride dentifrice was launched containing sodium hexametaphosphate, an advanced extrinsic whitening and anti-calculus agent in a low-water formulation, allowing the stannous fluoride and sodium hexametaphosphate to coexist in a stable single phase. This was marketed as Crest® Pro-Health™ or Oral-B® Pro-Expert™, depending on the region. It was the first dentifrice to deliver the full array of therapeutic benefits from stannous fluoride, with patient-desired tooth whitening and anti-tartar esthetic benefits, and was the first dentifrice with this broad range of protection to receive the American Dental Association Seal of Acceptance for reducing/preventing plaque, gingivitis, cavities, erosion, sensitivity, bad breath, and surface stain.[37] Global expansion was rapid.

Introducing Crest Pro-Health Smooth Formula

Seeking to maximize options within the stabilized stannous fluoride dentifrice portfolio, researchers at Procter & Gamble recently developed a new Crest Pro-Health product for patients who prefer a smooth texture dentifrice over the characteristic gritty texture of sodium hexametaphosphate, and/or who may not tolerate standard tartar control products well. This smooth texture version of Crest Pro-Health contains 0.454% stabilized stannous fluoride and zinc citrate as the anti-calculus agent. Zinc citrate inhibits calculus through the positively charged zinc ion (Zn^{2+}), which inhibits crystal growth by substituting for calcium in the crystal lattice of calcium phosphate. This interferes with the crystal formation and slows crystal growth.[38] The novel Crest Pro-Health smooth formula (Figure 3) also features a unique flavor/foaming experience to drive compliance, while delivering the full range of therapeutic and cosmetic benefits yielded by the original Crest Pro-Health formulation.

Figure 3. *Crest Pro-Health Smooth Formula.*

What the Research Shows

In this Special Issue of *The Journal of Clinical Dentistry*, extensive *in vitro* and clinical research evaluating the effectiveness of the novel Crest Pro-Health smooth formula is presented. The four randomized clinical trials reported herein were conducted independently among different populations and in diverse global locales (Figure 4).

Anti-Plaque Efficacy. The results of a 4-week, double-blind investigation at the University of Missouri-Kansas City are reported in this issue, wherein 120 adults were randomized to twice-daily unsupervised brushing with either Crest Pro-Health smooth formula or a 0.3% triclosan positive control dentifrice (Colgate® Total®; Colgate-Palmolive Company, New York, NY, USA).[39] Plaque was evaluated at baseline and Week 4 via the Rustogi Modified Navy Plaque Index, and the Crest Pro-Health Smooth Formula was found to produce significantly greater whole mouth plaque reduction compared to the Colgate control, with even higher relative benefits for the difficult-to-access approximal regions ($p < 0.0001$).

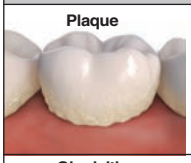
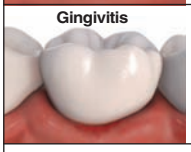
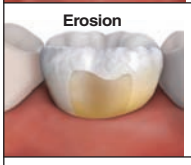
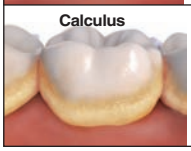
Outcome Measured	Dentifrice Test Products	Treatment Period	Benefit for Novel Stabilized Stannous Fluoride Dentifrice vs Control
Plaque 	Novel stabilized stannous fluoride Triclosan/sodium fluoride positive control	4 weeks	23.1% less whole mouth plaque 43.5% less interproximal plaque
Gingivitis 	Novel stabilized stannous fluoride Triclosan/sodium fluoride positive control	2 months	21.8% fewer bleeding sites
Erosion 	Novel stabilized stannous fluoride Sodium fluoride/potassium nitrate positive control	10 days	26.9% less erosion
Calculus 	Novel stabilized stannous fluoride Sodium fluoride negative control	3 months	21.7% less calculus

Figure 4. *Key clinical outcomes in this Special Issue.*

In a corresponding *in vitro* investigation reported in the same paper, the comparative acid production/glycolysis metabolic effects of the novel stabilized stannous fluoride and triclosan dentifrices were evaluated using the Plaque Glycolysis and Regrowth Model (PGRM). The stannous fluoride formula yielded significantly greater glycolysis inhibition versus the triclosan control ($p < 0.05$), demonstrating that the *in vitro* PGRM can be a valuable method of forecasting plaque inhibition in a clinical setting.

Anti-Gingivitis Efficacy. He and colleagues detail results of their two-month, double-blind, randomized and controlled clinical trial conducted at All Sum Research Center (Mississauga, Ontario, Canada) with 200 adults having pre-existing mild-to-moderate gingivitis.[40] Participants brushed at home for the study duration with their assigned dentifrice; either the novel 0.454% stabilized stannous fluoride smooth formulation or the 0.3% triclosan/NaF dentifrice positive control (Colgate Total). The Modified Gingival Index, the Gingival Bleeding Index, and the number of bleeding sites were used to evaluate gingival health status at both baseline and following two months of product use. Results showed the stannous fluoride smooth formula dentifrice provided significantly greater gingivitis reductions across all three clinical efficacy parameters compared to the triclosan positive control ($p < 0.0001$).

Acid Erosion Protection. Zhao, *et al.* provide a report in this Special Issue of a randomized and double-blind, three-period crossover trial conducted at the Fourth Military Medical University in Xi'an, China, wherein the enamel protection efficacy of the new 0.454% stannous fluoride smooth formula dentifrice was compared to that of marketed 0.5% potassium nitrate dentifrice (Sensodyne® Pronamel®; GSK, Brentford, United Kingdom).[41] This *in situ*, 10-day erosion model included the exposure of human enamel specimens in an intra-oral appliance to their respective assigned dentifrice, followed by an erosive challenge. At Day 10, the stannous fluoride dentifrice provided significantly greater erosion protection relative to the control dentifrice ($p < 0.03$).

Anti-Tartar Efficacy. The final paper shares results of a randomized, parallel group, double-blind clinical study conducted at Salus Research Inc. (Fort Wayne, IN, USA) to compare the tartar control benefits of the novel stannous fluoride dentifrice with zinc citrate versus a regular sodium fluoride negative control.[42] Participants were established calculus formers who received a baseline prophylaxis, and then brushed unsupervised for three months with their assigned toothpaste. Volpe-Manhold calculus examinations quantified the dentifrices' relative anti-calculus benefits, and revealed that the new stannous fluoride dentifrice provided significantly greater reductions in tartar at Week 12 compared to baseline versus the negative control ($p < 0.01$).

In the same paper, a separate *in vitro* investigation is reported utilizing the modified Plaque Growth and Mineralization Model (mPGM) to assess the respective plaque calcium levels after repeated dentifrice slurry treatments. Their findings demonstrate the new stannous fluoride toothpaste produced significantly greater calculus inhibition ($p < 0.05$) as compared to the negative control, providing evidence that mPGM can serve as a meaningful predictor of *in vivo* calculus formation.

Anti-Caries, Stain Removal, and Breath Odor Efficacy. In addition to the laboratory and clinical trial results presented in this Special Issue, research has shown that new Crest Pro Health smooth formula with 0.454% stabilized stannous fluoride provides other key therapeutic and cosmetic benefits.

Kennedy, *et al.* used an established *in vitro* pH cycling caries lesion progression model, which has been validated as a screening method to confirm anti-caries performance of fluoridated dentifrices,[43] to demonstrate that the anti-caries efficacy of the novel formula equaled that of a USP clinical standard, and was superior to a low-dose negative control.[44]

Friesen and colleagues compared the extrinsic stain removal ability of Crest Pro-Heath smooth formula to a positive control (Colgate® Total Whitening™, Colgate-Palmolive Company, New York, NY, USA) in a two-week, randomized and blinded clinical trial of 50 subjects with pre-existing stain.[45] An established examiner-graded index was employed to grade facial surfaces of test teeth following home use of the assigned test dentifrice. At Week 2, the novel stabilized stannous fluoride formula group had significantly less stain (whiter teeth), both overall and at interproximal surfaces, versus the positive control group ($p < 0.0001$).

Oral malodor reduction with use of the smooth texture stannous fluoride dentifrice relative to a sodium fluoride negative control was tested in a randomized and controlled, four-period crossover clinical trial evaluating Volatile Sulfur Compound (VSC) levels.[46] Compared to the negative control, mean VSC levels were significantly lower at overnight 24 hours, 48 hours, and daytime 51 hours with use of the stannous fluoride dentifrice ($p \leq 0.0099$), demonstrating its efficacy to improve halitosis.

Safety Profile

In the collective clinical trials reported in this Special Issue, the new 0.454% stabilized stannous fluoride smooth formula dentifrice was well-tolerated, with no safety-related subject withdrawals. Separately, Grender and colleagues assessed the oral desquamation profile of the novel dentifrice, which is a high water formula and therefore does not dry the oral mucosa. A meta-analysis was conducted of seven randomized and controlled clinical trials, both longer-term parallel group and shorter-term crossover design, where a total of 336 subjects used either stabilized stannous fluoride smooth texture formula or a regular non-tartar control fluoride dentifrice (Colgate Cavity Protection) daily.[47] Desquamation following dentifrice use was assessed by clinical examination or self-reported. There were no adverse event reports in the long-term studies. In the shorter-term trials, one or more instances of desquamation occurred in 5% and 4% of the stannous fluoride and non-tartar control dentifrice users, respectively. This difference was not significant between the dentifrice groups ($p = 0.543$).

Summary

Few individuals achieve perfect plaque removal with mechanical hygiene, and toothpaste plays a critical role delivering chemotherapeutic agents to improve oral health and provide cosmetic benefits. Procter & Gamble's focus on dentifrice innovation has led to a portfolio of stabilized, bioavailable stannous fluoride dentifrices due to stannous fluoride's unique range of benefits relative to other common fluorides, which only provide caries protection. The newly formulated Crest Pro-Health smooth formula was carefully designed to offer the broadest range and highest level of benefits (targeting caries, plaque, gingivitis, erosion, calculus, dentinal hypersensitivity, stain/whitening, and breath malodor) while optimizing the brushing experience and enhancing patient compliance. The research findings detailed in this Special Issue demonstrate the effectiveness of new Crest Pro-Health smooth formula to deliver significantly greater health and cosmetic benefits relative to various controls to improve patients' oral health.

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Conflict of Interest Disclosure statement: Dr. He and Dr. Farrell are employees of Procter & Gamble.

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Comparative Antiplaque Effect of Two Antimicrobial Dentifrices: Laboratory and Clinical Evaluations

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Abstract

- **Objective:** To compare the effect of a stannous fluoride dentifrice versus a triclosan-containing dentifrice on the reduction of plaque using *in vitro* and clinical models.
- **Methods:** Both investigations evaluated a novel 0.454% stabilized stannous fluoride dentifrice (Crest® Pro-Health™ smooth formula) versus a sodium fluoride/triclosan positive control dentifrice (Colgate® Total®). The *in vitro* evaluation utilized the Plaque Glycolysis and Regrowth Model (PGRM), wherein the metabolic effects (acid production/glycolysis inhibition) of the dentifrices were assessed on plaque biofilms grown on glass rods after three days growth and a single dentifrice treatment. Treatments were evaluated via analysis of variance, Student's t-test. The clinical trial was a four-week, single-center, randomized and controlled, double-blind, parallel group study, where 120 adults were randomized to one of the two dentifrices for use at home according to manufacturer's instructions. Plaque was evaluated at baseline and Week 4 with the Rustogi Modified Navy Plaque Index (RMNPI). Statistical analyses were via analysis of covariance.
- **Results:** *In vitro* PGRM: The stannous fluoride dentifrice provided 43.3% glycolysis inhibition compared to 27.5% for the triclosan control, and the pH decrease associated with acid production was significantly less for stannous fluoride (0.87) versus triclosan (1.11); $p < 0.05$. Clinical trial: One hundred eighteen (118) subjects completed the study with fully evaluable data. Both dentifrice groups demonstrated statistically significant ($p < 0.0001$) reductions in plaque at Week 4 compared with baseline, with the stannous fluoride dentifrice producing a significantly lower adjusted mean Week 4 plaque score ($p < 0.0001$) versus the triclosan positive control for whole mouth plaque (23.1% lower) and interproximal plaque (43.5% lower). Both dentifrices were well-tolerated.
- **Conclusion:** The stabilized stannous fluoride dentifrice provided statistically significant reductions in plaque glycolysis *in vitro* and plaque growth *in vivo* compared to the triclosan dentifrice. Results for both studies were consistent.

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Introduction

The most universally prevalent oral diseases, dental caries and periodontal disease, share a common precursor: dental plaque. Plaque, a biofilm of colonized bacteria, grows in both mass and virulence if undisturbed, as the microflora composition of stagnating colonies shifts with time from aerobic to anaerobic, with predominately gram-negative microorganisms.[1-6] Individual host factors influence the severity of the resulting gingival inflammation and bleeding that are the cardinal signs of gingivitis, and the risk of progression to periodontitis.[1] Similarly, individual immune factors and environmental variables (*e.g.*, sugar consumption) can affect one's susceptibility to caries with undisturbed plaque.[7]

Gingivitis is preventable, yet is extremely common in both youth and adults in the bulk of geographies and demographics.[8,9] A healthy periodontium, free of inflammation, is achievable via daily plaque removal using a manual toothbrush when all intraoral surfaces (including the lesser accessible approximal and gingival crevicular regions) are completely cleaned.[2,3,10] Research suggests this goal is difficult for many, with infrequent brushing, too-short duration of the brushing session, inability to reach every surface, and/or dexterity challenges as potential contributing factors.[11-13] It is theoretically possible to favorably alter patient behaviors/habits, but motivation and ongoing compliance must be high.[14] Alternatively, using more efficacious mechanical aids (*e.g.*, advanced-design manual or power toothbrushes) and incorporating chemotherapeutic products into the established oral hygiene regimen can render significant disease-fighting benefits without requiring challenging behavioral alterations in the patient's ingrained habits.

Chemotherapeutic adjuncts to tooth brushing for plaque control can reduce the pathogenicity of plaque microorganisms, and thus subsequently decrease the associated risk of gingival inflammation

and periodontal disease. Triclosan is one widely available broad spectrum bactericidal agent.[15,16] In commercially available dentifrice formulations, triclosan is typically at 0.3% concentration and combined with the copolymer Gantrez™ to augment effectiveness. A large scale review found antiplaque and antigingivitis benefits for this formulation when tested against a fluoride control dentifrice without triclosan/copolymer.[17]

A key attribute of an efficacious oral antimicrobial in any type of product is substantivity. Stannous fluoride is one of the most well-established oral over-the-counter chemotherapeutic agents, in part due to its known substantivity which lengthens the duration of its documented bacteriostatic and bactericidal actions.[18,19] Ramji and colleagues, for example, utilized *in vitro* salivary bacteria metabolic activity, Live/Dead assay, and *in vivo* tin retention trials to determine that a stannous fluoride dentifrice killed almost 100% of salivary microbes with a single exposure; it also produced active levels capable of bacterial metabolic inhibition 12 hours after exposure.[18] As a plaque control and antigingivitis agent, stannous fluoride dentifrices have demonstrated significantly greater efficacy relative to non-stannous controls in a large body of controlled clinical investigations of varying lengths, designs, and study populations.[20-25]

In 2005, a 0.454% stabilized stannous fluoride formulation was introduced as Crest® Pro-Health® dentifrice (Procter & Gamble Company, Cincinnati, OH, USA). This breakthrough formulation provided broad therapeutic benefits while also providing extrinsic whitening and tartar control efficacy.[21] The latest formulation innovation has produced a 0.454% stabilized stannous fluoride toothpaste that offers patients the option of a smooth texture with unique flavor and foaming experience without sacrificing the broad scope of clinically proven benefits in the original multi-benefit formulation.

Patients and clinicians are well-served by research on the comparative effectiveness of oral hygiene products to make informed recommendations. Testing products via distinct methodologies can provide added confirmation of their relative benefit profile. To assess the antimicrobial, antiplaque effectiveness of the new smooth formulation 0.454% stannous fluoride dentifrice when compared to a commercially available positive control triclosan dentifrice, two investigations, an *in vitro* evaluation and a clinical research trial, were conducted. In both investigations, the dentifrices compared were: Crest® Pro-Health® (smooth formula), The Procter & Gamble Company, Cincinnati, OH, USA; and positive control Colgate® Total® (0.3% triclosan/0.24% sodium fluoride, Colgate-Palmolive Company, New York, NY, USA.

Materials and Methods

In Vitro *Investigation*

The *in vitro* Plaque Glycolysis and Regrowth Model (PGRM) is an established method to efficiently evaluate the antiglycolytic activity of treatment compounds, by assessing their inhibitory effect on the metabolic pathways utilized by plaque microorganisms for acid and toxin production.

This investigation compared the antimicrobial response of the stannous fluoride and positive control triclosan dentifrices versus an assay negative control (Crest® Cavity Protection dentifrice, Procter & Gamble Company, Cincinnati, OH, USA). Following the PGRM protocol as described by White, *et al.*[26] and summarized here, plaque biofilms were prepared on glass rods using fresh pooled human saliva-spiked Trypticase Soy Broth (TSB) as growth media. Four rods were prepared per test group. After three days of growth, plaque specimens were treated a single time with 16.7% w/w dentifrice/water slurries for two minutes. Following rinsing, the plaque was then immersed in glycolysis media containing 0.5% sucrose in TSB, pH adjusted to 6.5. Plaque metabolism was monitored at 37°C until the assay negative control showed a change at approximately six hours, as monitored by change in pH indicator (Bromocresol Purple/Chlorophenol Red; Sigma Aldrich, St. Louis, MO, USA).

The metabolic effects of the test and positive control dentifrice treatments were evaluated via analysis of variance (ANOVA; Student's t-test using a 5% significance level) by measuring mean pH post-incubation to determine glycolysis inhibition, where a lesser decrease equated to greater treatment antimicrobial efficacy. The percent relative efficacy of the respective treatments was calculated versus the negative control as follows: (pH negative control minus pH test dentifrice) / pH negative control, multiplied by 100.

Clinical Trial

The clinical trial was a four-week, single-center, randomized and controlled, double-blind, parallel group investigation conducted in 120 adult volunteers aged 18 years and older and in good general health. The stannous fluoride test dentifrice and the triclosan positive control dentifrice were compared over the course of the trial for their relative plaque control efficacy. Before study initiation, the University of Missouri-Kansas City institutional review board approved the subject consent form and study protocol (IRB #16-176), and all participants provided verbal and written consent prior to enrollment.

Study entrance criteria required that the volunteers have at least 16 natural and scoreable teeth and a minimum whole mouth average plaque score of 0.5, as measured by the Rustogi Modified Navy Plaque Index (RMNPI).[27] Subjects who required antibiotic pre-medication before dental procedures, had taken antibiotics within two weeks of study inception, had fixed orthodontic appliances, or had significant oral neglect, rampant or untreated caries, and/or advanced periodontal disease were not eligible. Further, subjects who had a dental prophylaxis or elective dentistry, or used antibiotics during the course of the study could be withdrawn or have data excluded from the statistical analyses. Prior to screening and before all subsequent visits, subjects were required to stop oral hygiene, eating, drinking (except small sips of water up to 45 minutes pre-visit), chewing gum, and tobacco use within four hours before their appointment time.

At the baseline visit, subjects meeting all study entrance criteria received oral hard and soft tissue examinations. Dental plaque was disclosed using red dye (Gum® Red Cote®, Sunstar Americas Inc., Schaumburg, IL, USA) and swishing for one minute. Next, RMNPI plaque evaluations were conducted by an experienced clinical grader. All participants were then stratified by age, gender, and whole mouth average plaque scores prior to their random assignment via an encoded program to either the stannous fluoride dentifrice group or the triclosan control group; these assignments were conducted outside of the clinical examiner's presence for blinding purposes. Oral and written product use instructions were given, which specified that subjects were to brush at home over the four-week test period with the provided soft manual toothbrush (Oral-B Indicator®, Procter & Gamble Company, Cincinnati, OH, USA) and their assigned dentifrice according to each manufacturer's instructions: subjects in the

stannous fluoride dentifrice group were told to brush thoroughly for at least one minute twice daily (morning and evening); those assigned to the triclosan control dentifrice were directed to brush thoroughly, preferably after each meal or at least twice daily. All test dentifrices were overwrapped and packaged in identical test kit boxes to maintain subject blinding.

Four weeks after twice-daily unsupervised test dentifrice use, subjects returned for post-treatment evaluations. After a check of continuing eligibility, an oral hard and soft tissue evaluation was performed. Dental plaque was disclosed in the same manner as at baseline, and then RMNPI plaque examinations were conducted.

Clinical Efficacy Assessment

The RMNPI[27] was utilized to quantify pre-treatment (baseline) and post-treatment (four-week) dental plaque. On all 28 teeth, disclosed plaque was graded on nine sites per facial and lingual tooth surface, with a maximum 504 total sites (excluding 3rd molars, crowns, and surfaces with cervical restorations). Scoring was as follows: 0 = Absent; 1 = Present. A mean plaque index (MPI) was calculated for each subject by dividing the total number of tooth areas with plaque present by the total number of tooth areas scored on a whole mouth basis (areas A-I) and interproximally (D, F; Figure 1).

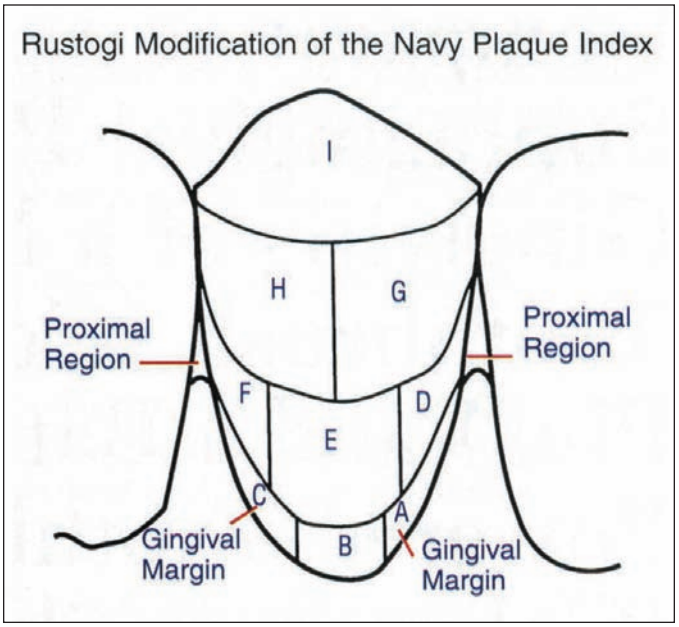

Figure 1. *The Rustogi,* et a.l[27] *Modification of the Navy Plaque Index. Disclosed plaque is scored in each facial and lingual tooth surface as present (1) or absent (0). The whole mouth is represented by areas A-I and interproximal (approximal) regions are D and F,*

Baseline subject demographic data were compared between test groups via ANOVA for age, Fisher's Exact Test for ethnicity, and chi-square for gender. The primary measure of efficacy was the Week 4 RMNPI. Summary statistics (*e.g.*, means, standard deviations, frequencies, etc.) of the demographic characteristics as well as RMNPI measurements were calculated for each treatment group and visit. For the Week 4 visit, the test groups were compared using the analysis of covariance (ANCOVA) method, with baseline as a covariate. The mean comparisons to baseline for each test group were investigated using paired-difference t-tests. Statistical tests were two-sided using a 5% significance level. Adverse event reports were summarized by treatment group.

Results

In Vitro *Investigation*

Table I summarizes the outcome of the PGRM investigation. The final mean pH of the stannous fluoride dentifrice-treated plaque after three days was 5.76, with a decrease in acid production pH of 0.867; the glycolysis inhibition (acid inhibition due to treatment) was 43.31%. For the triclosan dentifrice-treated plaque, the average final pH was 5.51 with a decrease in acid production pH of 1.111, signifying a 27.47% glycolysis/acid production inhibition. These collective results demonstrated that the antibacterial efficacy for the stannous fluoride dentifrice was significantly greater than for the triclosan dentifrice ($p < 0.05$).

Table I
In vitro Plaque Glycolysis and Regrowth Model (PGRM) Investigation Results

	Final pH (SE)	Acid Production Δ pH (SE)	% Glycolysis Inhibition (SE)	p-value[a]
Stannous Fluoride	5.76 (0.168)	0.867 (0.160)	43.31%	< 0.05
Triclosan control	5.51 (0.258)	1.111 (0.250)	27.47%	

SE = standard error; % = percentage; Δ = change
[a]Two-sided analysis of variance (ANOVA) for the between-treatment comparison

Clinical Trial

In the clinical study, 120 subjects were randomized at baseline to the two test groups, with 60 subjects per group. Two participants (one in each dentifrice group) withdrew voluntarily prior to study end; therefore 118 subjects (98%) finished the trial and had fully evaluable data. The age range of the study population was 18 to 71 years, with an average age of 42 years. Females comprised 78% of the randomized subjects and Caucasian (63%) and Black (28%) subjects accounted for 91% of the enrolled population. There were no statistically significant between-group differences for any of the baseline demographic variables ($p \geq 0.1691$; Table II).

Table II
Baseline Subject Demographics – Randomized Subjects

	Stannous Fluoride Test Dentifrice	Triclosan Positive Control	Overall
Characteristic	n = 60	n = 60	n = 120
Mean Age (SD)[a]	43.6 (14.93)	39.7 (15.29)	41.6 (15.17)
Age Range	20 - 71	18 - 70	18 - 71
Female (n, %)[b]	48 (80%)	45 (75%)	93 (78%)
Male (n, %)[b]	12 (20%)	15 (25%)	27 (22%)
American Indian[c]	1 (2%)	0 (0%)	1 (1%)
Asian Indian[c]	1 (2%)	0 (0%)	1 (1%)
Black[c]	15 (25%)	18 (30%)	33 (28%)
Caucasian[c]	41 (68%)	35 (58%)	76 (63%)
Hispanic[c]	2 (3%)	5 (8%)	7 (6%)
Multi-Racial[c]	0 (0%)	2 (3%)	2 (2%)

n = number of subjects; SD = standard deviation
[a]Two-sided ANOVA for the between-group mean age comparison (p = 0.1691).
[b]Two-sided chi square for the between-group gender balance comparison (p = 0.5119).
[c]Two-sided Fisher's Exact Test for the between-group ethnicity balance comparison (p = 0.2943).

Table III and Figures 2 and 3 outline the plaque clinical efficacy results. The test dentifrice groups were well-balanced in baseline mean RMNPI scores ($p > 0.7429$), where the study population's average pre-treatment whole mouth and interproximal RMNPI scores ranged from 0.51 to 0.76 and 0.79 to 1.0, respectively.

Table III
ANCOVA Treatment Comparisons[a]
Week 4 Rustogi Modified Navy Plaque Index (RMNPI) Results

	Baseline Mean (SD)	Week 4 Adjusted Mean (SE)	% Difference Between Dentifrices[b]	p-value[a]
Whole Mouth RMNPI				
Stannous Fluoride (n=59)	0.619 (0.051)	0.408 (0.007)	23.1%	< 0.0001
Triclosan control (n=59)	0.619 (0.051)	0.530 (0.007)		
Interproximal RMNPI				
Stannous Fluoride (n=59)	0.970 (0.052)	0.441 (0.018)	43.5%	< 0.0001
Triclosan control (n=59)	0.970 (0.048)	0.781 (0.018)		

SD = standard deviation; SE = standard error; % = percentage; n= number of Week 4 subjects
[a]Analysis of covariance with baseline as covariate; two sided p-value.
[b]In favor of stannous fluoride over triclosan positive control.

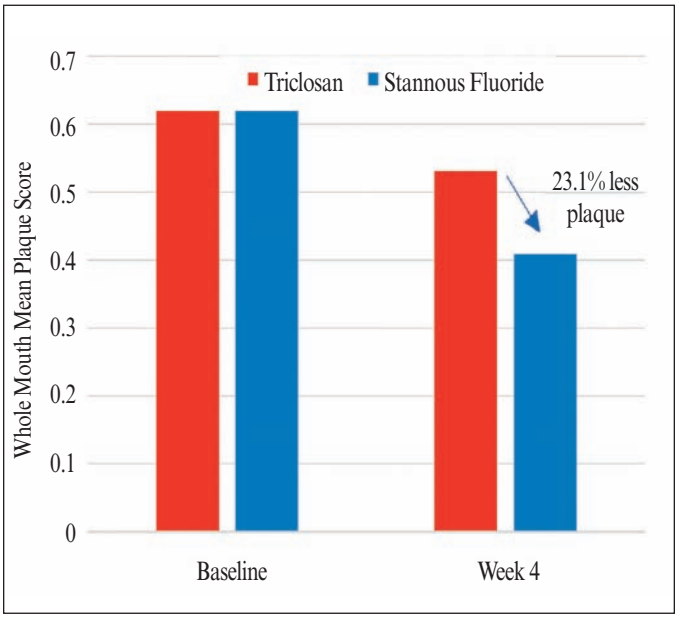

Figure 2. *Whole mouth mean RMNPI plaque scores. Week 4 values are the adjusted means. Stannous fluoride produced a 23.1% greater plaque reduction compared to triclosan ($p < 0.0001$).*

Both the stannous fluoride and triclosan control dentifrices significantly reduced whole mouth and interproximal plaque over the four-week test period compared to baseline ($p < 0.0001$). For whole mouth RMNPI, the stannous fluoride and triclosan control groups saw reductions in mean scores of -0.211 and -0.089, respectively, with Week 4 adjusted average scores of 0.408 for stannous fluoride and 0.530 for the triclosan control. The plaque score for stannous fluoride was 23.1% lower than that for the triclosan control ($p < 0.0001$; Table III and Figure 2).

RMNPI reductions in mean plaque scores versus pre-treatment

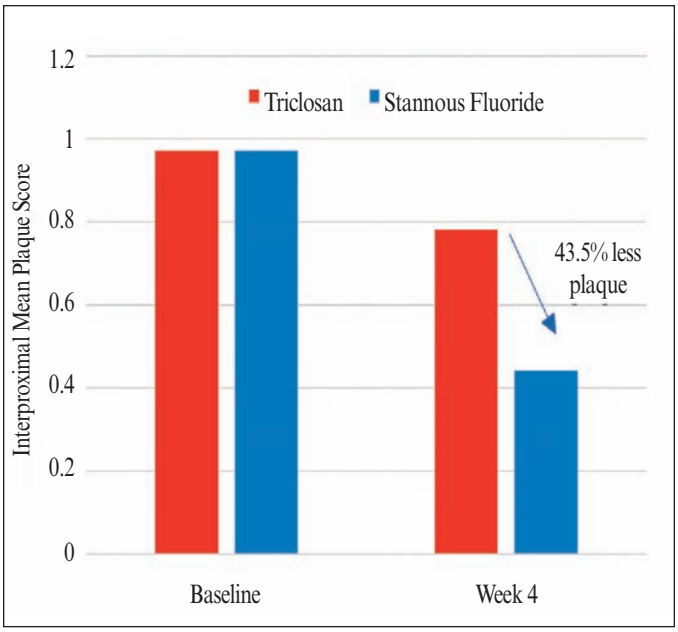

Figure 3. *Interproximal mean RMNPI plaque scores. Week 4 values are the adjusted means. Stannous fluoride produced a 43.5% greater plaque reduction compared to triclosan ($p < 0.0001$).*

in the interproximal region were -0.529 (Week 4 adjusted mean 0.441) for stannous fluoride, and -0.189 (Week 4 adjusted mean 0.781) for the triclosan control group. The Week 4 plaque score for stannous fluoride was 43.5% lower than that of the triclosan control toothpaste ($p < 0.0001$; Table III and Figure 3).

One subject in the stannous fluoride group experienced a mild, non-product-related adverse event (dysphagia), and did not discontinue study participation. Both dentifrices were well-tolerated.

Discussion

Pre-clinical *in vitro* models can be valuable in screening and assessing oral health antimicrobial ingredients and formulations. The *in vitro* PGRM has been shown to be an efficient and reproducible method for analysis of the relative glycolysis inhibition of dentifrices for both formulation screening and as a precursor to clinical testing.[18,26,28] In the *in vitro* investigation reported here, the stannous fluoride dentifrice performed significantly better than the triclosan positive control in the pH drop associated with acid production and glycolysis inhibition (43% compared to 27%; $p < 0.05$). The clinical trial results, based on manufacturer's usage instructions to mimic "real world" hygiene practices, similarly demonstrated clearly that the stannous fluoride dentifrice produced a significantly greater antiplaque benefit compared to the triclosan positive control. These clinical findings illustrate the usefulness of the *in vitro* PGRM methodology to differentiate between the two test products.

Both the stannous fluoride and triclosan control dentifrices provided significant antiplaque benefits compared with pre-treatment at Week 4 in the clinical study; however the stannous fluoride dentifrice yielded markedly better effectiveness across all regions of the dentition analyzed. The greater benefit for stannous fluoride compared to the triclosan control is in agreement with previous trials reported in the literature showing that stabilized stannous fluoride (SnF_2) provided greater plaque inhibition than triclosan.[23,25] In a six-week clinical trial of 114 subjects assigned to unsupervised usage of either a 0.454%

stabilized SnF_2 dentifrice or a 0.3% marketed triclosan control, the SnF_2 dentifrice generated 36.5% statistically significantly lower Week 6 mean whole mouth plaque (RMNPI), as well as statistically significantly lower scores versus the triclosan control in the gumline and interproximal regions.[23] Another method of plaque evaluation used digital imaging assessment of overnight plaque, and the 0.454% stabilized SnF_2 toothpaste similarly was tested against the 0.3% triclosan control in a three-week investigation, with SnF_2 producing a 17% lower adjusted mean for overnight plaque.[25]

The inclusion of the 0.3% triclosan dentifrice (Colgate Total) as the positive control in this clinical trial was predicated on triclosan's previously reported efficacy in clinical investigations of plaque inhibition and gingival health.[17,29,30] Triclosan's effects are largely bactericidal by disruption of bacterial cell wall membranes.[16,31,32] Due to triclosan's poor oral cavity retention when used in isolation, it is formulated in Colgate Total dentifrice with the polymer Gantrez, which enhances substantivity.[33] Recently, *in vitro* research investigating other means of antimicrobial mechanisms of action beyond plaque mass reduction evaluated plaque virulence.[34] Lipopolysaccharides (LPS) and lipoteichoic acids (LTA) inhibition assessments revealed that triclosan had no effect on LTA or LPS reactivity in the investigation; LTA and LPS are known to be associated with progressive periodontal disease. In contrast, in the same investigation, SnF_2 disrupted LTA and LPS reactivity and impeded the binding of LPS to cellular Toll-like receptors, with the authors concluding that stannous fluoride may prevent activation of cellular inflammatory processes.[34] These findings highlight another means by which stannous fluoride may confer plaque inhibition (and the associated clinically proven antigingivitis benefits), in concert with its established bacteriostatic and bactericidal actions and proven substantivity.[35,36] The multi-modal antiplaque qualities of stannous fluoride likely support its greater relative efficacy when contrasted to triclosan in the current and previously reported comparative laboratory and clinical trials.

The 43.5% lower Week 4 mean plaque score for the stannous fluoride dentifrice in the interproximal region compared to the triclosan control in this clinical investigation is particularly encouraging, given that plaque formation in this area tends to be more pronounced, likely because approximal tooth surfaces are notoriously more challenging to access and clean by the average brusher.[37,38] Over-the-counter chemotherapeutic products, like the novel smooth formula stannous fluoride dentifrice in this trial, can be an effective and simple means to control plaque even in more difficult-to-clean areas, and thus improve gingival health while providing numerous other oral health benefits in a single product.

Conclusion

The stabilized stannous fluoride dentifrice provided significant reductions in plaque glycolysis *in vitro* and plaque growth *in vivo* compared to the triclosan dentifrice. Results for both studies were consistent.

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Conflict of Interest: Dr. He, Dr. Zsiska, Dr. Schneiderman, Ms. Eusebio, and Ms. Farmer are employees of Procter & Gamble.

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Assessment of the Effects of a Novel Stabilized Stannous Fluoride Dentifrice on Gingivitis in a Two-Month Positive-Controlled Clinical Study

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Abstract

- **Objective:** The aim of this study was to compare the antibleeding/antigingivitis effectiveness of a newly formulated 0.454% stabilized stannous fluoride dentifrice and a marketed positive control triclosan-containing dentifrice in adults with mild-to-moderate gingivitis.
- **Methods:** This single-center, two-month, randomized and controlled, double-blind, parallel group clinical trial involved adults with pre-existing mild-to-moderate gingivitis. Baseline bleeding and gingivitis levels were assessed with the Gingival Bleeding Index (GBI) and Lobene Modified Gingival Index (MGI). Subjects were randomly assigned to either a new smooth formula 0.454% stabilized stannous fluoride test dentifrice (Crest® Pro-Health™) or a commercially available positive control 0.30% triclosan dentifrice (Colgate® Total®). Subjects brushed with their assigned dentifrice at home according to the manufacturer's instructions. At Month 2, subjects were re-evaluated for bleeding and gingivitis as at Baseline, with MGI and GBI evaluations.
- **Results:** Of the 200 subjects randomized to treatment, 197 completed the study and had fully evaluable data. At Month 2, both the stannous fluoride and triclosan control dentifrices produced statistically significant reductions ($p < 0.0001$) in the mean number of bleeding sites, MGI, and GBI compared to Baseline. Use of this 0.454% stannous fluoride dentifrice resulted in 22% fewer bleeding sites versus the positive control triclosan dentifrice ($p < 0.0001$). Similarly, after two months of brushing, the stannous fluoride dentifrice group showed statistically significant lower mean MGI and GBI scores than subjects using the triclosan positive control dentifrice ($p < 0.0001$). Both dentifrices were well-tolerated.
- **Conclusion:** Subjects brushing with a newly formulated stannous fluoride dentifrice had statistically significantly fewer bleeding sites and less gingivitis than those using a positive control triclosan dentifrice after two months.

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Introduction

Few conditions are more widespread than gingivitis. Worldwide, it is estimated that at least half of adults, and in some populations as many as 90%, have signs of gingivitis.[1,2] Yet afflicted individuals do not always recognize the characteristic gingival inflammation and bleeding upon brushing as indicative of disease, and may not fully grasp the vital role of effective oral hygiene in prevention, or the risk for more serious sequelae (periodontitis) if gingivitis is not arrested. Daily, thorough plaque removal via tooth brushing is universally recommended to patients by clinicians because gingivitis is nearly always plaque-induced; pathogenic microflora remaining after inadequate oral hygiene evolve in virulence within plaque biofilms to produce inflammation, gingival redness, swelling, and bleeding.[3-6] Barring intervention, chronic gingivitis may ultimately advance to periodontitis in susceptible individuals, bringing the risk for irreversible bone and tooth loss.[5]

It has been shown that individuals who possess the technical skill and motivation to remove all traces of plaque daily via standard manual tooth brushing are the exception rather than the rule, particularly in intraoral regions that are hard to access for comprehensive cleaning; many also do not brush long enough or frequently enough.[7-10] However, gingival health-promoting agents with proven chemotherapeutic benefits, such as decreasing plaque formation and altering the virulence of the plaque microflora, can fill the gap in an otherwise suboptimal plaque removal strategy, and provide significant benefits to both prevent and treat gingivitis. Over-the-counter chemotherapeutic adjuncts are available as mouthrinses and toothpastes, and may be incorporated into toothpastes in a multi-benefit product.

Two well-known and studied oral chemotherapeutics are triclosan and stannous fluoride.[11-16] Stannous fluoride produces bacteriostatic (plaque metabolism modulation) and bactericidal actions.[12] An impressive body of clinical research supports the effectiveness of stannous fluoride toothpastes in reducing plaque and gingivitis.[13-16] Studies have reported significantly greater gingival bleeding reductions with twice-daily brushing relative to non-stannous fluoride controls, including triclosan-containing dentifrices. Triclosan acts more through lysis of bacterial species and has been incorporated in dentifrices and marketed for plaque inhibition and antigingivitis benefits.

Stannous fluoride's significant efficacy in caries prevention has been recognized for decades.[17] In the last ten years it has experienced a notable resurgence in popularity as a first-line defense not only for cavity protection, but also gingivitis, erosion, breath malodor, and dentinal hypersensitivity reduction. The introduction of multi-benefit Crest® Pro-Health™ dentifrice (Procter & Gamble Company, Cincinnati, OH, USA) in 2006 marked the first stabilized 0.454%

stannous fluoride formulation to also provide anticalculus and extrinsic whitening benefits.[18] Researchers continue formula optimization to offer a portfolio of stabilized stannous fluoride dentifrice products with diverse brushing experiences to meet different patient preferences and motivate compliance.

Recently, a novel stabilized stannous fluoride dentifrice has been introduced with a smooth texture and unique flavors and foaming. This innovative formulation, marketed as Crest Pro-Health (Smooth Formula), continues to provide the full range of clinically proven oral health and esthetic benefits.[19-23] Additionally, patients who experience oral desquamation from tartar control dentifrice use should find this novel smooth-texture tartar control formula more appealing, as its desquamation profile is comparable to a regular, non-tartar control fluoride dentifrice.[24] To compare its gingivitis-fighting effects when compared to a marketed triclosan dentifrice positive control, a two-month randomized and controlled clinical investigation was conducted in adults with mild to moderate gingivitis.

Materials and Methods

In this two-month randomized, double-blind, parallel group clinical study at a single clinical center, the antigingivitis effectiveness of a new 0.454% stabilized stannous fluoride dentifrice relative to a commercially available 0.30% triclosan positive control dentifrice was investigated in generally healthy adult volunteer subjects with mild-to-moderate gingivitis. Prior to study inception, the subject consent form and study protocol were reviewed and approved by the BRCL institutional review board (#16064-14:17:9015-06-2016).

Eligibility for enrollment was determined at a screening visit, where volunteers provided written informed consent and a medical history, and received clinical oral hard and soft tissue examinations and a gingivitis evaluation. To qualify for participation, subjects needed a minimum of 16 natural, scoreable teeth, and were required to show evidence of gingivitis with 10 to 50 bleeding sites via the Gingival Bleeding Index (GBI)[25] and a Lobene Modified Gingival Index (MGI)[26] score ranging from 1.75 to 2.3. Individuals who were pregnant or lactating, required antibiotic pre-medication prior to dental procedures, had fixed orthodontic appliances, or had significant oral neglect and/or advanced periodontal disease were not eligible. Additionally, any of the following within the two weeks preceding the screening visit and thereafter throughout the trial precluded study enrollment and/or ongoing participation: antibiotic/anti-inflammatory/anticoagulant medication use; antigingivitis/antibacterial oral care product use (*e.g.*, chlorhexidine); or dental prophylaxis. Prior to screening and before all subsequent visits, subjects were required to stop oral hygiene, eating, drinking (except small sips of water up to 45 minutes pre-visit), chewing gum, and tobacco use within four hours before their appointment time.

At the Baseline visit, subjects who met all study entrance criteria received pre-treatment oral hard and soft tissue evaluations, the MGI examination, and the GBI evaluation from an experienced examiner.[27-29] They were then stratified via whole mouth mean GBI and MGI sum scores, gender, and smoking status, and were randomly assigned using an encoded program to one of the two test dentifrice groups: 1) Crest® Pro-Health™ (Smooth Formula); Procter & Gamble Company, Cincinnati, OH, USA; or 2) the marketed positive control Colgate® Total® (0.3% triclosan/0.24% sodium fluoride); Colgate-Palmolive Company, New York, NY, USA. To ensure double-blinding, test group assignments and product dispensing were conducted in a protected area distinct from that of clinical examinations, and the dentifrice tubes were overwrapped and packaged in identically appearing test kit boxes.

Subjects conducted their first brushing onsite at the Baseline visit. Thereafter, all test dentifrice use was unsupervised at home for the two-month study test phase with the assigned dentifrice and an Oral-B® Indicator (Procter & Gamble Company, Cincinnati, OH, USA) soft manual flat trim toothbrush. Subjects used the assigned dentifrice per manufacturer's usage instruction.

After two months of assigned dentifrice use, subjects were recalled. Continued eligibility was assessed and post-treatment safety and efficacy evaluations were conducted in the following order: oral hard and soft tissue evaluation, MGI, and GBI examinations. Test dentifrice safety was monitored via a visual assessment of the oral cavity soft and hard tissues with a standard light, dental mirror, and gauze, and examining the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips, perioral area, dentition, and restorations.

Two well-established clinical indices were utilized in this trial by an experienced dentist and grader to assess gingivitis and gingival bleeding. The Lobene Modified Gingival Index (MGI)[26] assesses gingivitis inflammation by scoring six gingival areas (distobuccal, buccal, mesiobuccal, mesiolingual, lingual, and distolingual) of all scoreable teeth using a scale of 0-4 as follows:

0 = normal (absence of inflammation);
1 = mild inflammation (slight change of color, little change in texture) of any portion of, but not the entire marginal or papillary gingival unit;
2 = mild inflammation of the entire gingival unit;
3 = moderate inflammation (moderate glazing, redness, edema and/or hypertrophy) of the marginal or papillary gingival unit; and
4 = severe inflammation (marked redness and edema/hypertrophy, spontaneous bleeding or ulceration) of the marginal or papillary gingival unit.

The GBI[25] evaluation was performed next by the clinical grader. Here the gingiva is lightly air-dried and a periodontal probe is gently moved around the gingival crevice. Each of six gingival areas (distobuccal, buccal, mesiobuccal, mesiolingual, lingual, and distolingual) of the scoreable teeth is probed in this manner, waiting approximately 30 seconds before recording the number of gingival units which bleed, as follows:

0 = absence of bleeding after 30 seconds;
1 = bleeding observed after 30 seconds; and
2 = immediate bleeding observed.

The number of bleeding sites for each subject and visit was calculated by collapsing GBI scores of 2 to a value of 1.

Baseline subject demographic data were compared between test groups via analysis of variance (ANOVA) for age and Fisher's Exact Test for gender and ethnicity. The primary efficacy endpoint of interest was the number of bleeding sites. The efficacy statistical analyses were based on the sum of the GBI scores, the number of bleeding sites, and whole mouth average MGI scores. A paired-difference t-test was

used for comparisons to Baseline for each efficacy index and within each treatment group. Using analysis of covariance (ANCOVA) with the corresponding Baseline score as a covariate, a statistical between-treatment group post-treatment comparison, along with the improvement after two months for the number of bleeding sites, was conducted at Month 2. Improvement at Month 2 was calculated as a positive value of Baseline minus Month 2.

Up to 200 subjects were allotted for enrollment, with 95 subjects completing in each test group providing a minimum of 80% power to detect a mean between-group difference of 1.56 for GBI using two-sided testing with a 5% significance level).

Results

At Baseline, 200 subjects were randomized to the two test groups: 100 subjects were assigned to the stannous fluoride dentifrice and 100 were assigned to the positive triclosan control. A total of 197 subjects (99%) were evaluable; 2 subjects dropped from the study due to non-treatment-related reasons and one subject was excluded due to non-compliance. Subjects' age ranged from 18 to 76 years, with a mean age of 47.9 years. Seventy-three percent (73%) of randomized subjects were female. With respect to ethnicity, Caucasian, Black, and Asian Indian subjects comprised 57%, 26%, and 11% of the study population, respectively. The two test dentifrice groups were well-balanced for age, gender, and ethnicity ($p \geq 0.2590$; Table I).

Table I
Baseline Subject Demographics – Randomized Subjects

	Stannous Fluoride Test Dentifrice	Triclosan Positive Control	Overall
Characteristic	n = 100	n = 100	n = 200
Mean Age (SD)[a]	48.7 (10.60)	47.0 (10.64)	47.9 (10.63)
Age Range	22 – 76	18 – 65	18 – 76
Female (n, %)[b]	71 (71%)	75 (75%)	146 (73%)
Male (n, %)[b]	29 (29%)	25 (25%)	54 (27%)
Asian Indian	12 (12%)	10 (10%)	22 (11%)
Black	22 (22%)	30 (30%)	52 (26%)
Caucasian	57 (57%)	56 (56%)	113 (57%)
Other	9 (9%)	4 (4%)	13 (7%)

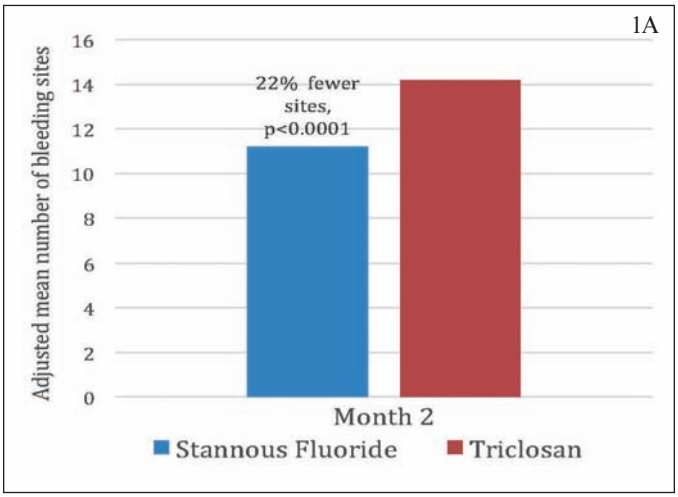
n = number of subjects; SD = standard deviation
[a]Two-sided ANOVA for the between-group mean age comparison (p = 0.259).
[b]Two-sided Fisher's Exact Test for the between-group gender balance comparison (p = 0.633).
[c]Two-sided Fisher's Exact Test for the between-group ethnicity balance comparison (p = 0.439).

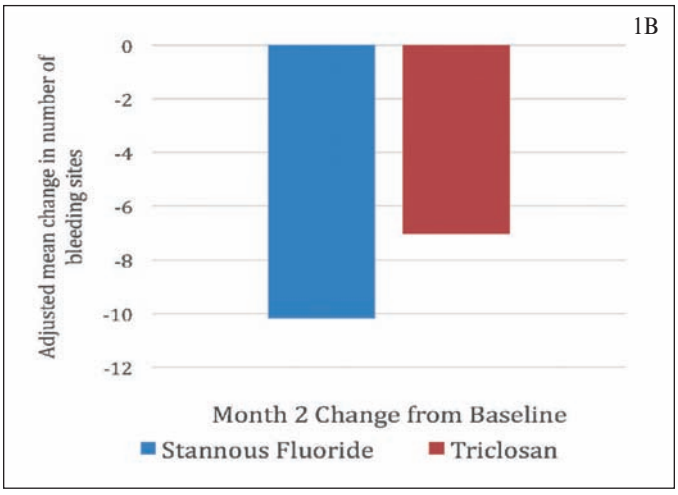
There were no significant pre-treatment between-group differences in the various measures of gingival health ($p \geq 0.4164$), with the number of Baseline bleeding sites averaging 21.32 (range 10 to 49) in the overall study population, the mean MGI averaging 2.06 overall (range 1.87 to 2.29), and the GBI scores ranging from 10 to 68, with a mean of 22.88 overall in the study population. After two months of assigned dentifrice use, when compared with Baseline, subjects in both test groups had statistically significantly ($p < 0.0001$) fewer bleeding sites on average (Table II). Adjusted means (SE) at Month 2 were 11.21 (0.261) and 14.33 (0.262) for the stannous fluoride and triclosan dentifrice groups, respectively, representing significantly fewer bleeding sites for the stannous fluoride dentifrice ($p < 0.0001$; Figures 1A and 1B).

Table II
Summary Statistics of Number of Bleeding Sites, GBI, and MGI

	Baseline Mean (SD) (n = 199)	Month 2 Mean (SD) (n = 197)	Mean Improvement (SD) (n = 197)	p-value[a]
Number of Bleeding Sites				
Stannous Fluoride dentifrice	20.70 (10.22)	10.80 (7.17)	9.97 (4.59)	< 0.0001
Triclosan positive control	21.95 (11.40)	14.74 (8.17)	7.26 (4.31)	< 0.0001
Gingival Bleeding Index				
Stannous Fluoride dentifrice	22.44 (12.61)	11.20 (7.81)	11.27 (6.58)	< 0.0001
Triclosan positive control	23.32 (13.75)	15.38 (9.31)	8.01 (5.79)	< 0.0001
Modified Gingival Index				
Stannous Fluoride dentifrice	2.06 (0.09)	1.82 (0.15)	0.24 (0.11)	< 0.0001
Triclosan positive control	2.06 (0.10)	1.89 (0.14)	0.16 (0.10)	< 0.0001

GBI = Gingival Bleeding Index; MGI = Modified Gingival Index; n = number of subjects; SD = standard deviation.
Improvement = Baseline minus Month 2
[a]Comparison versus baseline using a two-sided paired-difference t-test.





Figures 1A and 1B. *A) Adjusted mean number of bleeding sites at Month 2. B) Adjusted mean change in number of bleeding sites at Month 2. Adjusted means were from ANCOVA analysis.*

Mean GBI scores at Baseline were 22.44 for the stannous fluoride and 23.32 for the triclosan positive control test groups (Table II). After two months of brushing, both dentifrices yielded statistically significantly lower mean GBI scores versus Baseline ($p < 0.0001$; Table II). Adjusted mean Month 2 GBI scores were significantly lower ($p < 0.0001$) in the stannous fluoride group (11.48) as compared to the triclosan positive control group (15.10).

The mean MGI score at Baseline was 2.06 for both groups (Table II). At Month 2, both dentifrices provided significant MGI reductions versus Baseline ($p < 0.0001$; Table II). Adjusted mean Month 2 MGI scores were statistically significantly lower ($p < 0.0001$) in the stannous fluoride group (1.82) versus the triclosan positive control (1.90), respectively.

No adverse events were reported or observed, indicating both dentifrices were well-tolerated.

Discussion

Gingival bleeding should warrant close attention and a proactive treatment plan as it is a key symptom of gingivitis.[5,6] Common risk assessment tools for periodontal disease include bleeding as a risk factor.[30] It follows that a chief measure of success for a chemotherapeutic antigingivitis product is the prevention and reduction of gingival bleeding. In this two-month investigation of the comparative antigingivitis abilities of a new smooth formula, stabilized 0.454% stannous fluoride dentifrice and a marketed 0.30% triclosan/0.24% sodium fluoride toothpaste with known benefits (Colgate Total) in adults with mild-to-moderate gingivitis, the stannous fluoride dentifrice markedly reduced gingival bleeding, showing statistically significantly better efficacy than the triclosan positive control with 22% fewer bleeding sites.

These results corroborate and mirror the findings of three previous clinical trials assessing the impact of stannous fluoride dentifrices on gingival health. In two independent randomized clinical trials with analogous study designs to that reported here, after two months of use the stabilized stannous fluoride dentifrice provided significantly greater reductions in the number of gingival bleeding sites when compared to the 0.30% triclosan/copolymer negative control: 34% fewer bleeding sites ($p < 0.0001$) in a study population of 150,[31] and 35% fewer sites ($p < 0.0001$) in a population of 200.[27] A longer investigation of stabilized stannous fluoride dentifrice compared to the same triclosan/copolymer comparator dentifrice was conducted by Archila and colleagues.[13] In that six-month trial with a Baseline prophylaxis and combination of supervised and unsupervised brushing, the stannous fluoride dentifrice produced 27% less gingival bleeding ($p < 0.001$) as compared to the triclosan/copolymer control at Month 6.

Both study dentifrices in the current trial, each containing a proven antigingivitis chemotherapeutic agent, generated statistically significant reductions in gingivitis and gingival bleeding with two months' brushing. Stannous fluoride achieves its clinical efficacy in part via bacteriostatic, and to a lesser degree, bactericidal actions. A series of independent investigations on a stabilized stannous fluoride dentifrice by Ramji, *et al.* were illustrative, including an *in vitro* Live/Dead assay and salivary bacteria metabolic activity studies, along with *in vivo* Plaque Gycolysis and Regrowth Model and 12-hour tin retention studies.[12] The results demonstrated that the stannous fluoride dentifrice killed up to 99% of salivary microbes 16 hours following a single exposure, produced significant plaque acid and plaque regrowth reductions, and provided ongoing total soluble tin (a marker for active stannous fluoride) at levels sufficient for salivary bacterial metabolic inhibition 12 hours post-treatment. Taken together, these results confirmed stannous fluoride's substantivity and long-lasting antimicrobial activity, undergirding its proven clinical chemotherapeutic effectiveness.

Independent of its antibacterial actions, other mechanisms by which stannous fluoride treats and prevents gingivitis have been demonstrated. Laughlin, *et al.* tested the impact of stannous fluoride on host and bacterial pro-enzymes involved in different inflammatory pathways via MMP, ICE, and R-gingipain assays, and found that stannous fluoride inhibited several pro-inflammatory enzymes and produced greater gingipain inhibition than triclosan; they concluded that stannous fluoride demonstrated prevention of tissue destruction and direct anti-inflammatory activity.[32] In another *in vitro* study, Haught and colleagues[33] recently evaluated the ability of triclosan and stannous fluoride to inhibit lipopolysaccharides (LPS); that is, bacterial endotoxins highly prevalent on root/gingival surfaces of those with periodontitis. They also evaluated lipoteichoic acids (LTA), gram-positive bacteria cell wall components associated with inflammation and acute infection. The results showed that stannous fluoride, but not triclosan, interfered with LTA and LPS reactivity in dye assays. Similarly, stannous fluoride, but not triclosan, inhibited LPS binding to cellular Toll-like Receptor 4, thereby rendering plaque less virulent.

Triclosan's effects against gingivitis are primarily bacteriocidal.[34,35] Anti-inflammatory activity has also been demonstrated and was described by Panagakos and colleagues.[36] Triclosan is most efficacious when formulated with the copolymer Gantrez, and triclosan/copolymer dentifrices have been clinically shown to provide plaque and gingivitis reductions.[11,37] This reported *in vivo* antigingivitis efficacy was the basis for the use of Colgate Total as the positive control in the current clinical trial, together with its inclusion in previous studies comparing stannous fluoride and triclosan dentifrice. As in those trials, the newly formulated stannous fluoride dentifrice had significantly greater efficacy than the triclosan positive control for all gingival health measures, including number of bleeding sites, MGI, and GBI. Stannous fluoride's multi-modal mechanisms of action targeting gingivitis and long-lasting effects against pathogenic bacteria can be presumed to account for the greater benefits seen here and in previous trials with typical home product use when compared to the triclosan control.

The presence or absence of gingival bleeding is an objective marker of gingival health that is trackable outside of clinical research by the patient's clinician. As shown in this well-controlled investigation, regular home use of a dentifrice with a clinically proven chemotherapeutic agent such as triclosan or stannous fluoride can measurably reduce bleeding and improve gingivitis in as little as eight weeks. The new smooth variant 0.454% stabilized stannous fluoride dentifrice in this study produced sizably greater, significant bleeding reductions when compared to the triclosan control dentifrice. Considering the numerous other oral health benefits also afforded by this stannous fluoride dentifrice (breath malodor, plaque and calculus control, hypersensitivity protection), simply incorporating it into the existing daily oral hygiene routine is an excellent strategy for securing better gingival health.[38]

Conclusion

Subjects brushing with a newly formulated stannous fluoride dentifrice had significantly fewer bleeding sites than those using a positive control triclosan dentifrice after two months, and therefore should be recommended as the dentifrice to patients with gingivitis to reduce bleeding and improve periodontal health.

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Conflict of Interest: Dr. He and Ms. Eusebio are employees of Procter & Gamble.

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A Randomized Clinical Trial to Measure the Erosion Protection Benefits of a Novel Stabilized Stannous Fluoride Dentifrice versus a Control Dentifrice

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Abstract

- **Objective**: The aim of this investigation was to assess the erosion protection ability of a novel stabilized stannous fluoride (SnF_2) dentifrice and a control sodium fluoride dentifrice (NaF) using a well-credentialed human *in situ* model.
- **Methods**: A novel smooth texture 0.454% stabilized SnF_2 dentifrice (Crest® Pro-Health™ smooth formula) and a 0.23% NaF marketed control dentifrice with 5% potassium nitrate (Sensodyne® Pronamel®) were compared in a 10-day, single center, randomized, controlled, double-blind, two-treatment, three-period crossover *in situ* clinical trial. Subjects wore a mandibular buccal appliance fitted with eight enamel specimens for approximately six hours over the course of each study day. Twice daily, subjects brushed the lingual surfaces of their teeth for 30 seconds while wearing the appliance, then swished with their assigned treatment toothpaste slurry for 90 seconds under the supervision of clinic staff. Erosive acid challenges with a citric acid-containing beverage (commercial orange juice) were done four times each day.
- **Results**: The SnF_2 dentifrice provided 26.9% greater erosion protection relative to the NaF dentifrice at Day 10 ($p < 0.03$). Adjusted means of enamel surface loss at Day 10 were 9.117 μm for the SnF_2 dentifrice and 12.471 μm for the NaF marketed control.
- **Conclusion**: These results demonstrate the stabilized SnF_2 dentifrice offered greater protection over the NaF dentifrice against the initiation and progression of dental erosion.

(J Clin Dent 2017;28(Spec Iss B):B17–20)

Introduction

In previous decades, there have been significant increases in the consumption of acidic drinks, including soda, fruit juices, and sport drinks.[1] Such beverages, if taken in excess, can promote erosion of the tooth surface, defined as the loss of tooth substance by chemical processes not involving bacterial action.[2] The incidence of dietary dental erosion is becoming an ever-increasing problem and has led to greater scientific awareness of the condition within the dental community.[3]

One obvious method to reduce dental erosion is through the delivery of enamel protection agents to exposed tooth surfaces that are most susceptible to erosive acid damage. Oral care products used in daily oral hygiene represent a convenient delivery system for agents that provide erosion protection. Stabilized stannous fluoride in dentifrice formulations has been shown to offer high levels of protection against both the initiation and progression of dental erosion. *In vitro*[4-8] and human *in situ* erosion clinical trials,[8-12] both of which are routinely used to assess comparative product performance, have demonstrated significant differences between stannous-based fluoride dentifrices and those containing other agents with respect to their ability to provide erosion prevention benefits.

The development of dentifrice products is something of an art, with many products going through an evolutionary process as researchers evaluate product efficacy and consumer reaction to the brushing experience (*e.g.*, flavor, foaming, etc.). The original stannous fluoride dentifrices of the late 1950s were effective against caries, but they were relatively unstable formulations that did not enjoy a long-term shelf life.[13] Technology improvements led to more stable formulations of high levels of antimicrobial efficacy in the 1990s. Later, these formulations evolved to include stain and calculus prevention technology, providing both therapeutic and esthetic benefits.[14] The most recent evolution of the stabilized stannous fluoride dentifrice technology includes a "smooth" formula paste that contains stannous chloride as a sacrificial ion to stabilize stannous fluoride. The new formula has unique flavors and foaming action to further improve its in-use experience, providing patients with even more options in the stabilized stannous fluoride dentifrice portfolio.

The current trial evaluated the erosion protective effects of the novel smooth version of a stabilized stannous fluoride dentifrice compared to a marketed sensitivity dentifrice formulated with NaF and potassium nitrate, using a variation of well-established *in situ* clinical models.[8,10-12]

Materials and Methods

This single-center, double-blind, randomized, two-treatment, and three-period crossover study was a variation of the previously published method of Hooper, *et al.*[8] The study was designed and managed in compliance with the principles of Good Clinical Practice. Ethical approval was granted by the Beijing Health Tech Research Co. Ltd Institutional Review Board under study number CSD2016153. After receiving both verbal and written information concerning the study, each participant gave signed and witnessed consent to their participation.

Prior to the start of the study, healthy adult participants were instructed to refrain from using any prescription or non-prescription oral care products that were not assigned as test articles for the duration of the study. Participants were also instructed to refrain from receiving an oral prophylaxis or any other elective dental procedure over this same timeframe.

At screening, participants were provided with a non-treatment 0.243% sodium fluoride (1100 ppm fluoride) marketed dentifrice (Crest® Cavity Protection dentifrice, The Procter & Gamble Company, Guangzhou, China) and flat-trim manual toothbrushes (Crest® Wairouneigang manual toothbrush, The Procter & Gamble Company, Guangzhou, China) for use at home until the follow-up visit. Participants were required to use these products in place of their normal oral care products, twice per day (morning and evening) for the duration of the study, including treatment days and weekends.

Each subject presented for three study periods and was randomized to one of two treatment sequences (ABB, BAA), receiving one of the two marketed dentifrice products each period:

- Crest® Pro-Health dentifrice (smooth formula): 0.454% stabilized stannous fluoride (The Procter & Gamble Company, Cincinnati, OH, USA)
- Sensodyne® ProNamel® dentifrice (control): 0.243% NaF with 5% potassium nitrate (GlaxoSmithKline Consumer Healthcare, Moon Township, PA, USA)

Each study period was comprised of 10 treatment days, which were conducted only on weekdays (Monday through Friday). In order to maintain blinding, study participants and the study laboratory personnel who conducted the surface profilometry measurements were not aware of the specific identity of the assigned test dentifrices during the treatment periods.

On each treatment day, subjects brushed their teeth at home in their usual manner, using a non-treatment fluoride toothpaste and a regular soft manual toothbrush supplied at the screening visit. Subjects then attended the clinical trials unit where they collected their custom-fit lower buccal acrylic intra-oral appliance, fitted with eight enamel samples, and placed it in the mouth, four on the left and four on the right side (Figure 1). Subjects wore the appliance for approximately six hours in total over the course of each study day. While wearing the appliance, subjects brushed the lingual surfaces of their teeth for 30 seconds, and swished with their assigned treatment toothpaste slurry for 90 seconds, twice a day, under the supervision of clinic staff.

The erosive challenge occurred with the appliance in the mouth. Subjects were required to sip 25 mL of orange juice (Uni-President Enterprises (China) Investment Co., LTD) over a timed minute, swishing it around their mouth, then spitting out. This was repeated 10 times so that a total of 250 mL of orange juice was exposed to the enamel samples over a 10-minute period. Four erosive challenges were made each treatment day. On Day 10, the enamel samples were measured for tissue loss using a calibrated non-contact surface profilometer. Measurements were taken at baseline, prior to the start of the study, and at the end of treatment Day 10. Fresh enamel samples were placed in the intra-oral appliance at the beginning of each treatment period.

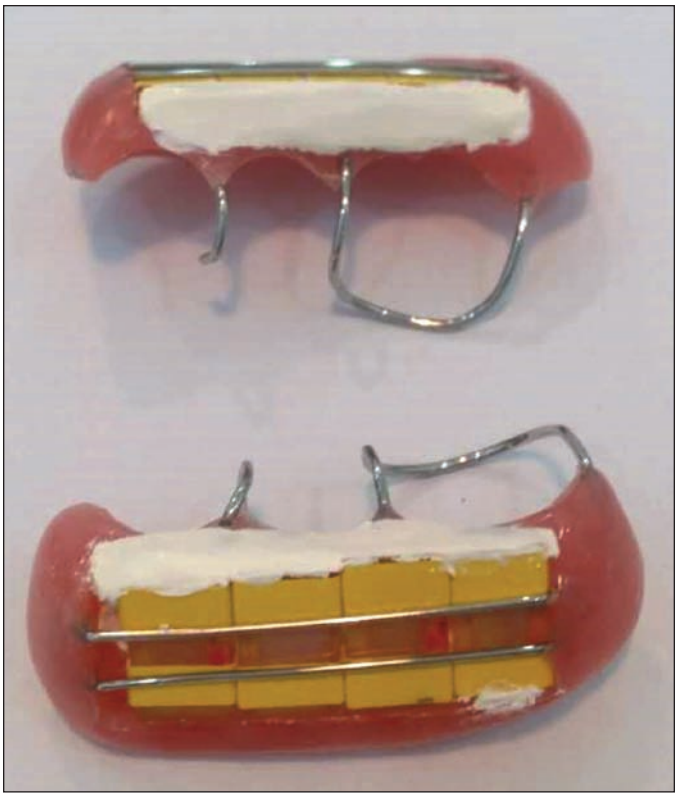

Figure 1. *Oral appliances fitted with 8 enamel specimens.*

The appliances (containing the enamel samples) were removed for one hour over lunch and also overnight until the next day. When removed at these times, the appliances were stored in a "moist pot" (a jar containing a damp cotton pad, moistened with water). The appliances were also disinfected in mouthrinse (Listerine® Cool Mint, Johnson & Johnson, New Brunswick, NJ, USA) twice daily at the start of the treatment day and upon removal from the mouth at the end of each treatment day.

Specimen Preparation

The enamel samples were prepared at the School of Stomatology, the Fourth Military Medical University (Xi'an, China). Caries-free human third molars, donated by adult patients, were used for the enamel samples. After extraction, teeth were cleaned using standardized procedures and stored in a thymol solution until use.

Tooth crowns were sectioned into 1 mm slices to produce the enamel samples. Each enamel sample was then prepared using a series of grinding and polishing procedures to produce smooth specimens with a high surface luster. Specimens were polished flat to have profile tolerances of ± 0.1 µm at baseline readings. Specific procedures for preparing enamel specimens for use in intraoral model studies have been discussed in greater detail in other publications.[8,10-12]

Two baseline readings of each enamel sample were taken using a non-contact profilometer. Samples were masked with PVC tape on either side of a 2–3 mm wide window of enamel. Each enamel sample was identified with a unique number on the reverse side of the enamel sample using a permanent marker for post-treatment analysis (Figures 2a, 2b).

Figure 2a. *Enamel samples with identified figure on the reverse side.*

Figure 2b. *Masked samples with identified figure on the tape.*

Statistical Efficacy Analyses

The primary measure of efficacy in this study was dental erosion that had occurred, measured by profilometry, over the 10-day study period. For each subject, treatment period, and visit, the average of four erosion measurements was calculated using two replicate measurements from each of two enamel sections. A general linear mixed model was used to compare treatments with a statistical model that included period, side, and treatment as fixed effects, and subject as a random effect. From the statistical model, estimated means on the natural log scale were back-transformed using the exponential function (emean) to obtain the estimated medians or 50th percentiles on the original scale (μm), along with the associated standard errors and/or 95% confidence intervals (CI). Statistical comparisons were two-sided at a 5% significance level.

Results

Twelve participants were randomized to a treatment sequence and all completed the study. They ranged in age from 25–62 years with a mean age of 36.3 years. Since each subject wore an appliance containing eight specimens, a total of 96 enamel specimens were measured in each study period, analyzed in sets of eight.

At Day 10, the stabilized stannous fluoride dentifrice demonstrated a statistically significant ($p < 0.03$) 26.9% better protection against erosion versus the control dentifrice with estimated enamel loss means (SE) of 9.117 μm (2.002) for the stannous fluoride dentifrice and 12.471 μm (2.002) for the control dentifrice (Figure 3). Both dentifrices were well tolerated. No significant adverse events were reported.

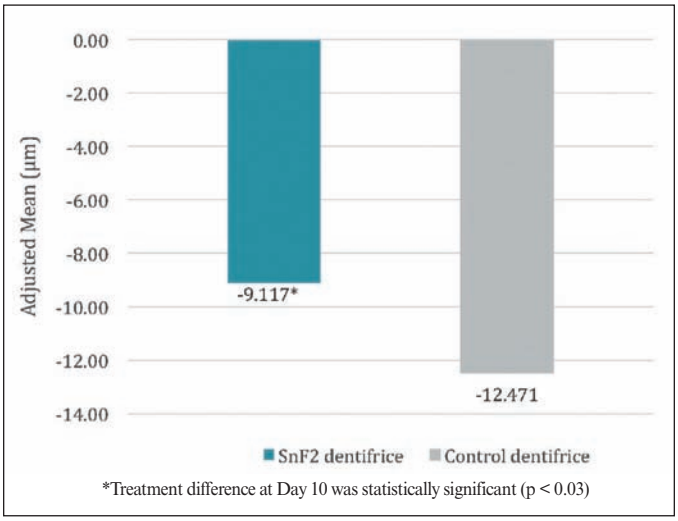

Figure 3. *Enamel loss at Day 10.*

Discussion

Human *in situ* clinical models provide an excellent means for assessing the relative erosion protective efficacies of oral care products. When conducted using well-designed, short-term study protocols, these models are able to show significant differences between products with varying degrees of effectiveness.[8-12] The ability to show statistical differences within such a short period of time enables clinical assessments of relative effectiveness without exposing the natural teeth to excessive erosive conditions. This is particularly important in the study of dental erosion, which is essentially an irreversible process.[1,2] Although some studies have demonstrated remineralization of erosively softened lesions *in vitro*[15,16] or *in vivo*,[17,18] the likelihood of strengthening erosive lesions *in vivo* to the point that they will be permanently restored is unlikely given the multitude of daily physical challenges these tooth surfaces receive. Once the enamel is erosively softened *in vivo*, it is likely that these softened surfaces will eventually be lost to erosive tooth wear.[19] As a result, the development of products capable of preventing softening and tooth surface loss is a more preferred approach than attempts to remineralize and restore damaged tooth mineral.

The protocol used in the current study is a variation of models used by Hooper and others in previous studies.[8,10-12] In most of the previous studies, palatal appliances were used. In this study, as in a study by Bellamy and colleagues,[10] mandibular buccal appliances were used. Studies were conducted at different locations with diverse subject populations. These studies have demonstrated the superiority of stabilized stannous fluoride dentifrices to inhibit the initiation and progression of dental erosion relative to various controls.[8-12]

The protective advantages against dental erosion offered by stannous fluoride relative to sodium fluoride are likely due to differences in mechanism of action. Dentifrices formulated with sodium fluoride rely on the ability of the fluoride active alone to strengthen the tooth surface and help prevent dental erosion. Stannous fluoride deposits

a stannous-rich, acid-resistant barrier, which has been shown to remain on the tooth surface for several hours after treatment,[20] acting in a sacrificial manner to neutralize an acid challenge. The barrier layer most likely contains either stannous fluorophosphate or stannous oxide compounds,[21] either of which would provide significantly higher resistance to an erosive acid challenge compared to a precipitate of calcium and fluoride, which is likely the type of deposit delivered from dentifrices containing sodium fluoride and potassium nitrate.

Dental erosion is considered an etiological factor for dentin hypersensitivity, another common condition resulting from the loss of tooth minerals that cover the dentin tubules.[22] The smear layer formed by stabilized stannous fluoride to protect against erosion also helps to inhibit sensitivity. The tin-rich barrier occludes open dentinal tubules, reducing fluid flow within the tubule and thereby alleviating pain.[23] The ability of stannous fluoride dentifrice to reduce sensitivity has been demonstrated in numerous clinical trials.[24-26] The evidence demonstrating the unique range of benefits offered by stannous fluoride, including protection from erosion, caries, and sensitivity, is important for dental professionals when making home care recommendations to patients. The latest "smooth texture" version of the stabilized stannous fluoride dentifrice, with unique flavors and brushing experience, offers patients yet another option to obtain the broadest range of therapeutic and esthetic benefits a dentifrice can provide.

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Conflict of Interest: Dr. He, Ms. He, Ms. Cheng, and Dr. Chen are employees of Procter & Gamble. Dr. Zhao reports no conflicts of interest.

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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

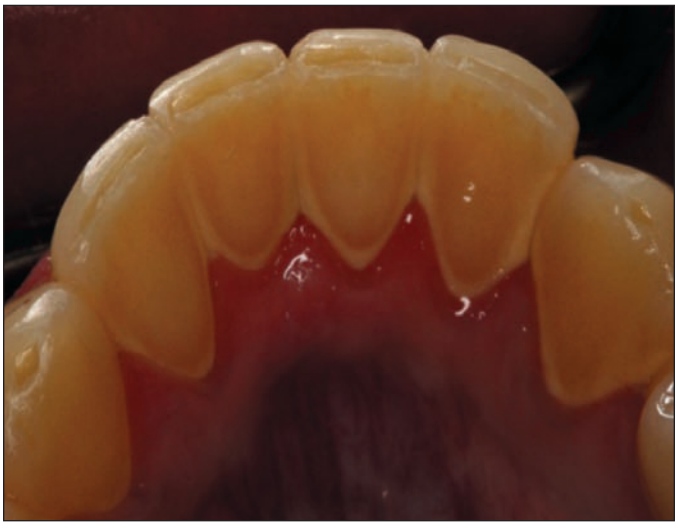


Figure 1. *Supragingival calculus tends to be greater in areas adjacent to the salivary glands, such as on the mandibular anterior lingual tooth surfaces.*

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 microorganisms.[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

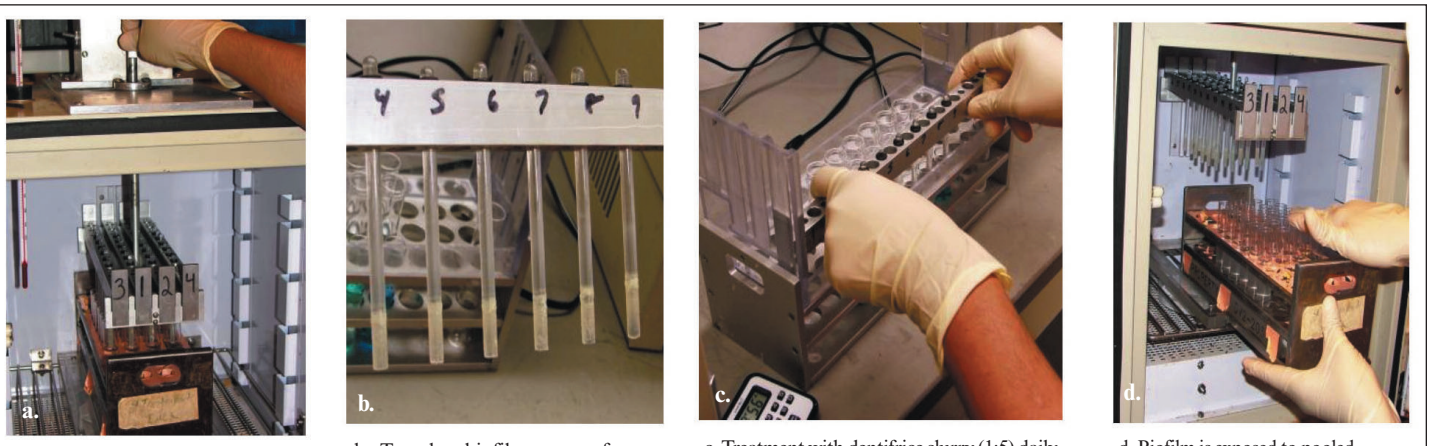
In Vitro *Investigation*

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.*

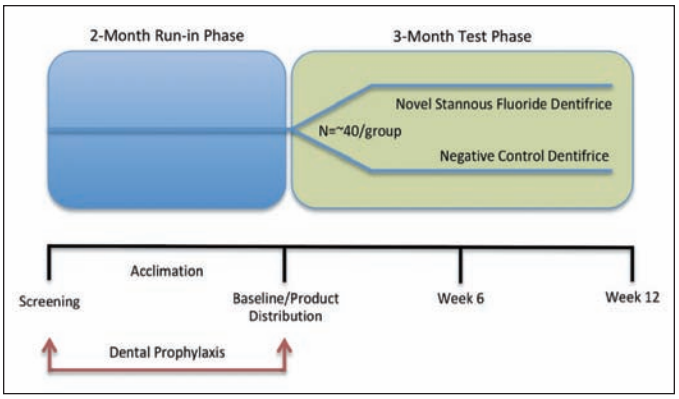


Figure 3. *The clinical trial design incorporated a two-month run-phase, followed by a three-month test phase.*

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)

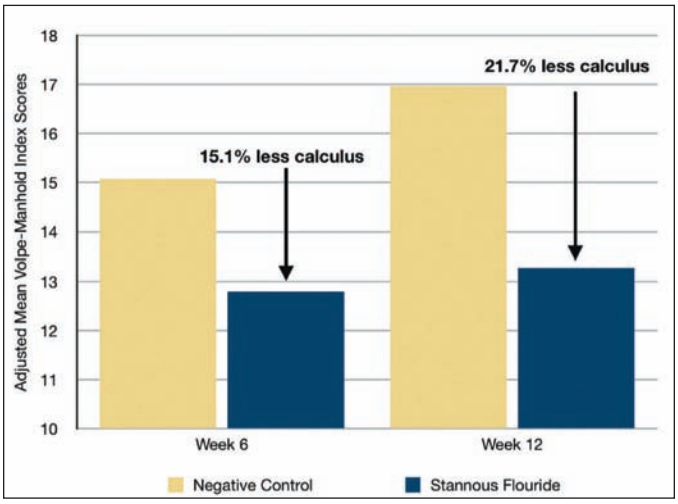


Figure 4. *The stannous fluoride dentifrice provided a calculus inhibition benefit compared to the negative control toothpaste at both Weeks 6 ($p = 0.052$) and 12 ($p = 0.006$).*

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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