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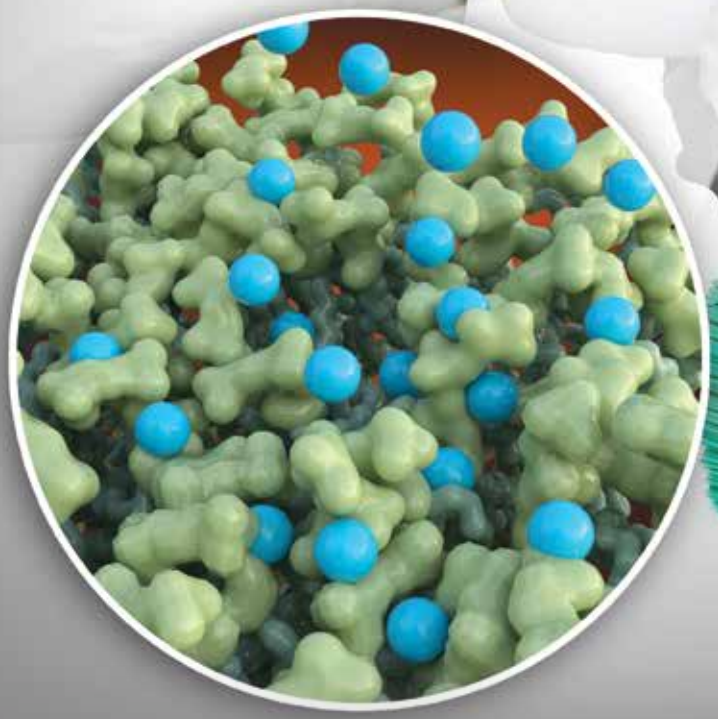

Active Prevention

Unlocking the Power of Stannous Fluoride: A New Dimension in Oral Health

Research includes: Antibacterial, Plaque/Gingivitis, Dentin Hypersensitivity, Oral Malodor, and Stain Removal In Vitro and Clinical Efficacy

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CONTENTS

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Advanced Oral Health Protection: A Multifunctional Dentifrice Designed to Prevent Oral Diseases and Conditions

Cassiano Kuchenbecker Rösing, DDS, MSc, PhD

In 1948, the United Nations General Assembly in Paris declared that a standard of health was a universal human right.[1] There is no reason to suggest that this affirmation should not include oral health as well. Oral health is a human right!

Cassiano Kuchenbecker Rösing, DDS, MSc, PhD

Oral diseases are a public health problem that impair quality of life and generate increased costs and demands.[2] The World Economic Forum reported this year that almost half the world's population suffers from oral diseases that impact daily life and subject the population to a higher risk of systemic health issues.[3] It is time for radical policy action. Governments, industries, academic institutions, and scientific organizations must help change the burden of oral diseases.[4] Concerned about the lack of attention to oral health, a historic resolution was adopted by the World Health Assembly in 2021 stating that oral health should be firmly embedded within the noncommunicable disease agenda and that oral healthcare interventions should be included in universal health coverage programs.[5]

The most prevalent oral diseases—caries and periodontal diseases—are considered noncommunicable chronic illnesses that share risk factors with other diseases of the body and are strongly related to lifestyle and behaviors.[6] Dental caries and periodontal diseases, which are largely preventable, stem from the accumulation of dysbiotic biofilms frequently worsened by modern-day dietary choices, such as increased sugar consumption, frequent snacking, tobacco use, and acidic/alcoholic beverages.[7] In the United States more than 40% of adults present with some level of periodontitis, which evidence suggests is likely the result of poor oral healthcare, brushing irregularity, and ineffective dentifrice.[8,9] Modern dietary habits and lifestyle choices pose continuous challenges to the oral cavity, necessitating a protective approach that can preserve the delicate balance of the oral ecosystem and prevent disease.

Preventive programs based on change in lifestyle—notably, increasing the quality of oral hygiene—reduce the occurrence of caries and periodontal diseases and lead to diminished tooth loss.[10] More recently, preventive practices have also been demonstrated in periodontitis treatment.[11] However, balancing dysbiotic biofilms and plaque control as it is performed by the majority of the population has limitations. It is of interest to create mechanisms to compensate for difficulties in plaque control. Even in clinical studies, with strict protocols, it is important to warrant good standards of plaque control.[12] It is well understood both in clinical practice and in research that there are clear limitations for good standards of plaque control. This is not only the reality for physically impaired individuals and an aging population, but plaque control for many people is suboptimal due to difficult-to-reach areas that demand dexterity. Thus, there is an opportunity to provide tools to compensate for such difficulties. While plaque debridement through toothbrushing and enamel protection with fluoridated toothpaste are fundamental, evidence suggests that suppression of biofilms with an antibacterial toothpaste can provide the preventive care needed to control caries development.[9] Active prevention, through the use of an advanced antibacterial fluoride toothpaste that not only offers cavity protection but also fights gingivitis, can play a pivotal role in reducing this burden by impeding the onset and progression of oral diseases and the development of potential systemic health issues linked to poor oral hygiene. Importantly, antibacterial toothpastes have the advantage of offering continued efficacy between brushing.

Breakthrough Technology

Stannous fluoride (SnF_2) has been studied in depth and widely indicated for antibacterial, antiplaque, anticaries, and antigingivitis effects.[13-15] However, the antiplaque and antigingivitis clinical efficacy of SnF_2 is dependent on maintaining the 2+ oxidation state of stannous.[14-16] In the past, this has proven to be a challenge

since SnF_2 readily hydrolyzes and oxidizes in aqueous solutions to form Sn^{4+} ions, which are therapeutically inactive.[16-19] Many approaches to prolong the stabilization of stannous in a 2+ oxidation state (Sn(II)) have been attempted with deleterious effect. For example, preventing hydrolysis through the removal of water reduces product desirability and is difficult to process; adding more Sn(II), such as stannous chloride, increases Sn(II) availability but also increases the potential for tooth stain and compromises the taste; and processing the formula under N_2 removes oxidation pathways but has limited long-term storage stability.[20]

The development of a multifunctional SnF_2 toothpaste stabilized by nitrate and phosphates (SNaP) is a breakthrough technology with the potential to improve oral health in individuals and populations. This technology increases stability and bioavailability of SnF_2 with the potential of increasing the quality of plaque control.[21] This advanced formula allows more flexibility to offer benefits, such as various flavors, foam, and esthetics, that people expect from mainstream dentifrices. This approach also aligns with the contemporary trend toward minimalism in consumer goods, which prioritizes ingredient transparency and efficacy. New formulations must not only demonstrate clinical efficacy but also garner positive consumer feedback, which drives compliance. This dual focus enables the product to be effective in preventing oral diseases and also appealing to patients, helping facilitate better oral health outcomes on a broad scale. The development of a therapeutic toothpaste like SNaP is informed by user trends (eg, increased interest in health), scientific research, and global oral health shifts reported by authoritative bodies such as the World Health Organization, World Dental Federation, Pan American Board of Oral Health Examiners, and European Federation of Periodontology. This helps ensure that the product meets consumer demands while addressing the current oral health context by delivering a highly efficacious product with the potential to reduce the incidence of oral diseases and, consequently, the need for more invasive and costly dental treatments.

In short, the effectiveness of active prevention in fighting oral diseases lies in being able to empower individuals, maximize efficacy, and improve adherence to oral hygiene practices. The development of advanced toothpaste formulations that are both effective and appealing to consumers can significantly reduce the burden of oral diseases, contributing to the overall health and well-being of the population. Empowering patients to maintain their oral health is crucial, as it fosters a sense of personal responsibility and proactive behavior toward oral hygiene. The use of advanced toothpaste formulations such as the one presented in this special issue can help redefine the standards of preventive dentistry and public health. The aim of the present article is to review the evidence supporting this dentifrice and help translate the results of research into clinical practice.

Summary of Supporting Evidence

The studies presented in this special issue support the development of a multifunctional toothpaste. These studies broadly support different potential benefits of Colgate Total®. The studies were delineated under contemporary research paradigms, and diseases/conditions were approached with clinical research that follows all laboratory work that supports the principles and mechanisms of action.

Antibacterial Effects

The antimicrobial potential of SNaP, including both in vitro and in vivo methods, was conceived under the concept that dysbiosis needs to be controlled and that biofilm is a natural colonizer of the human body.[22] In this sense, one should not aim at eliminating the biofilm, but maintaining the biofilm in a symbiotic relation with the host. Elimination of oral biofilms would have negative outcomes, such as the occurrence of opportunistic infections (eg, fungal infections). A dysbiotic biofilm initiates an inflammatory process in the gingival tissues, leading to the occurrence of gingivitis and even periodontitis. In this sense, helping to disrupt oral biofilms with chemical agents is of extreme interest. A symbiotic relationship with oral biofilms is, therefore, an aim in preventive dentistry.

Active prevention, through the use of an advanced antibacterial fluoride toothpaste that not only offers cavity protection but also fights gingivitis, can play a pivotal role in reducing this burden by impeding the onset and progression of oral diseases and the development of potential systemic health issues linked to poor oral hygiene. Importantly, antibacterial toothpastes have the advantage of offering continued efficacy between brushing.

Therapeutic approaches for controlling oral biofilms may include the use of chemical agents. For this purpose, oral biofilm derived from saliva of healthy individuals was used and cultured on hydroxyapatite discs. This biofilm was transferred and toothpaste was applied to it. Treatment consisted of SNaP as the test treatment, a non-antibacterial toothpaste containing 0.24% sodium fluoride and 5% potassium nitrate as a negative control, a toothpaste containing 0.454% stannous fluoride stabilized with sodium gluconate (SnF_2 + SG) as a positive control, and no treatment. Antibacterial performance was measured by monitoring bacterial metabolic function for bacterial respiration and glycolysis. The results clearly demonstrated bacterial suppression by SNaP as compared to controls, showing the potential of SNaP to reduce bacterial load in the mouth. This

result has the potential of helping reduce oral problems that are related to dysbiotic biofilms.

In the same article, the authors also clinically tested the antibacterial potential demonstrated in the in vitro model.[22] A randomized controlled clinical trial was performed comparing SNaP to a non-antibacterial toothpaste. Bacterial samples were collected from the tongue, cheek, dental plaque, gingival tissues, and saliva. The results were analyzed 12 hours post-brushing at 2 and 4 weeks. In all timepoints and for all bacterial samples, statistically significant differences were observed when test and control groups were compared, in favor of SNaP. The clinical experimental model confirmed the results from the in vitro methods. Therefore, it was demonstrated that the SNaP technology prevents regrowth of the biofilm for prolonged periods after brushing. These results support the potential benefits in prevention and treatment, being considered an active preventive approach, of the most prevalent oral diseases with a dentifrice containing SnF_2 stabilized with nitrate and phosphates.

Antigingivitis and Antiplaque Properties

Since gingival diseases are prevalent in clinical dentistry, the SNaP dentifrice was clinically tested on a plaque and gingivitis model.[23] Such a model is used to give support for clinical effects of oral health products, demonstrating potential in prevention and treatment. This study was a randomized controlled clinical trial performed over 6 months, with intermediate analysis at 3 months. Plaque was assessed by Quigley-Hein plaque index (Turesky modification) and gingivitis was assessed by the Löe and Silness gingival index. SNaP was compared to a regular fluoride-containing toothpaste. The results were stratified to all tooth surfaces and also specifically for interproximal surfaces, which are the most difficult-to-reach areas and where oral diseases tend to be more pervasive. To provide a more clinically relevant result, severity of both plaque and gingival indices were calculated.

At all timepoints, for all analyses, SNaP performed better than the regular fluoride-containing toothpaste. For example, in terms of gingival severity index, which is extremely relevant clinically, a 90% difference was obtained comparing both groups at 6 months. In gingival interproximal areas (remembering that individuals did not floss during the period), the difference between SNaP and the control group was 35.3%. These results need to be understood in a perspective of disruptive knowledge: a chemical agent helps the user control the dysbiotic biofilm in difficult-to-reach areas, which is a clear limitation of sole mechanical plaque control. This was demonstrated over a 6-month period, which is a considerable amount of time to prove the dentifrice's effect in preventing occurrence of gingival inflammation.

Hypersensitivity Relief

Dentin hypersensitivity is a common oral condition that impairs quality of life, as it is one of the chronic pains of the body. Dentin hypersensitivity occurs after the exposure of dentin due to both gingival recession and the loss of dental hard tissues such as enamel. The presence of fluoride has an active potential in preventing dental erosion and treating dentin hypersensitivity. In this sense, stabilized SnF_2 not only is a preventive agent but also serves as a therapeutic approach. The potential of SNaP dentifrice in alleviating dentin hypersensitivity was tested both in vitro and in vivo.[24] By means of confocal microscopy, occlusion of dentin tubules was measured in extracted human teeth treated either with SNaP or with a regular fluoride-containing toothpaste. Dentin specimens were brushed with the designated toothpaste for 30 seconds and this procedure was repeated 5 times. The results demonstrated an occlusion of dentin tubules of 86% and 35% for SNaP and the control dentifrice, respectively.

In a randomized controlled clinical trial the antisensitivity effect was tested, comparing SNaP (test group), a potassium nitrate desensitizing dentifrice (positive control), and a non-desensitizing sodium monofluorophosphate dentifrice (negative control). Analyses were performed at baseline and days 1, 3, and 7. Both air blast and tactile stimuli were used. The results indicate that SNaP significantly reduced hypersensitivity pain after 1, 3, and 7 days. After 7 days, individuals that used SNaP dentifrice exhibited an additional reduction in tactile hypersensitivity of 79.8% as compared to the positive control group, and 90.2% as compared to the negative control group with tactile stimulus. These values were 47.1% and 47.9%, respectively, when air blast stimulus was considered.

Malodor Control

A dentifrice is also useful in the management of oral malodor. Halitosis is a prevalent condition that impacts quality of life and causes social restraint. It primarily originates in the mouth and is related to the presence of dysbiotic biofilms. It is also considered an esthetic problem, because it impairs social relationships. The understanding of this point can also improve compliance with its management. The antimicrobial effect of SNaP, mitigating bacterial load on oral surfaces, including hard and soft tissues, is crucial in managing halitosis. The effect of SNaP dentifrice on oral malodor was tested by means of a randomized controlled clinical trial compared with a regular fluoride-containing toothpaste.[25] The trial lasted 3 weeks and organoleptic measurements were used to assess malodor. After 3 weeks of trial, a reduction of 32.7% on overnight malodor score reduction as compared to baseline was observed for the SNaP group while the control group exhibited a 9.4% reduction. Additionally, 85.7% (42 out of 49) of the subjects who brushed with SNaP toothpaste entered the pleasant breath zone (organoleptic score ≤ 5), while none from the control group achieved such a result.

Additionally, a consumer test of preference for flavor and freshening attributes was performed. Participants preferred the flavor and freshening attributes of the new SNaP toothpaste over the in-market formulation of Colgate Total. This demonstrates the dentifrice meeting patient preferences and beliefs as part of evidence-based healthcare.

Stain Reduction

Esthetic outcomes have grown considerably in clinical dentistry. In addition to halitosis, patients indicate that tooth stain

reduction and whitening play an important role in personal confidence. Esthetic demands are growing worldwide and the use of dentifrices has been included in the management of esthetic conditions. The effect of SNaP dentifrice on extrinsic tooth staining was tested in a randomized controlled clinical trial.[26] The comparative group was a regular fluoride-containing toothpaste. The trial lasted 6 weeks and demonstrated additional stain reduction by SNaP dentifrice of 24.3% at 3 weeks and 39.1% at 6 weeks compared to the control.

In all studies supporting the clinical effects of SNaP dentifrice, no adverse effects were reported.

Conclusions

The concept of active prevention with SNaP dentifrice technology is supported by studies of different design, addressing its capability of acting against the cause of the most prevalent oral conditions. Studies performed in different research centers around the world have clinically demonstrated its effect on plaque, gingivitis, dentin hypersensitivity, tooth staining, and oral malodor, among other oral conditions. SNaP toothpaste is clinically proven to support oral health with high-quality evidence and should be part of the standard of oral hygiene care, helping to prevent and treat the most prevalent oral health conditions.

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DISCLOSURE

The author acts as an independent consultant to Colgate Palmolive Co.

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The Evolution of Colgate Total®: A New Era Stabilized by Nitrate and Phosphates

Lisa M. Manus, PhD; Carl P. Myers, PhD; Robert D'Ambrogio, BS; Gokul V. Govindaraju, PhD; Guofeng Xu, PhD; Yun-Po Zhang, PhD, DDS (Hon); and James G. Masters, PhD

Abstract: Effective and accessible oral care strategies, like the use of a multi-benefit, antimicrobial toothpaste, are a key tool in preventive public health. For over 30 years, Colgate Total toothpastes have represented a gold standard in multi-benefit toothpastes to help fight bacteria and provide whole-mouth care. This review introduces the next generation of Colgate's research and development featuring stannous fluoride (SnF_2) stabilized by nitrate and phosphates. The uniqueness of this engine is detailed through a review of SnF_2 oral benefits, the historic challenges associated with SnF_2 toothpastes, and the advantages that this chemistry can bring to patients seeking multi-benefit oral care. With this novel technology, a new balance in efficacy, stability, and streamlined design enables flexible formulation and customized user experiences inspired by key therapeutic areas.

The tipping point between oral health and disease is influenced by a variety of inherent and environmental risk factors. The health of the oral cavity can be impacted by genetics, diet, accessibility to routine dental care, perceptions of and past experiences with dental professionals, socioeconomic status, and daily oral hygiene practices.[1-4] Despite scientific advances in fundamental knowledge and the development of therapies to help reduce risk factors, caries and periodontal disease remain as two of the most dominant oral health issues globally. Currently, approximately 3.5 billion people around the world have experienced at least one form of these diseases in their lifetime.[5,6] Accessible, affordable, and effective interventions that help attenuate the risk of oral diseases are key. Many common oral health conditions are to a great extent preventable and can be reduced through appropriate oral care.[7-9] In addition to routine dental exams, daily oral hygiene with a fluoride toothpaste featuring an antimicrobial control agent can provide an important action to help prevent the risk of oral diseases.[10-12]

A root cause of many common oral issues is the growth of pathogenic bacteria in the mouth (Figure 1).[13] Directly or indirectly, these microorganisms can cause damage to hard and soft tissues in the oral cavity, potentially leading to advanced states of disease over time. Bacteria can be present throughout the mouth in planktonic form or within diverse biofilms anchored to an oral surface like the teeth, tongue, cheeks, and gums. If an oral biofilm is left undisturbed, it grows and matures into a biofilm that can be harmful to one's oral health. These mature oral biofilms serve as a safe haven for bacteria and enable them to exchange nutrients, form communication networks with each other to defend against threats, and build structures to resist stress from the environment.[14,15] If these biofilms are not reduced in mass or vitality (and this growth is coupled with additional risk factors favoring disease), they continue to proliferate and a shift in the composition of the microflora may occur that favors pathogenic bacteria.[16-18] An abundance of pathogenic bacteria and their byproducts can be a root cause of many common oral health issues given their ability to release toxins that irritate gums, produce volatile sulfur compounds promoting oral malodor, and generate acids that break down dental hard tissues.

To a certain degree, some oral bacteria and biofilms can be removed through mechanical actions such as brushing with a non-antimicrobial fluoride toothpaste and flossing. However, a lack of consistent compliance to a comprehensive oral hygiene routine can limit the ability of these actions to effectively fight bacteria.[19] Intervention with antimicrobial agents has been clinically proven to reduce oral bioburden significantly by helping control bacteria growth and mitigate bacterial byproducts, even between brushings.[20-22] Antimicrobial agents can also help fight bacteria in areas

of the mouth where toothbrushing may be limited, such as interproximally and on soft-tissue surfaces like the buccal mucosa, tongue, and gums. Therefore, targeting bacteria, a root cause of many common oral health issues, with an antimicrobial-based dentifrice provides a more viable route to help in oral disease prevention.

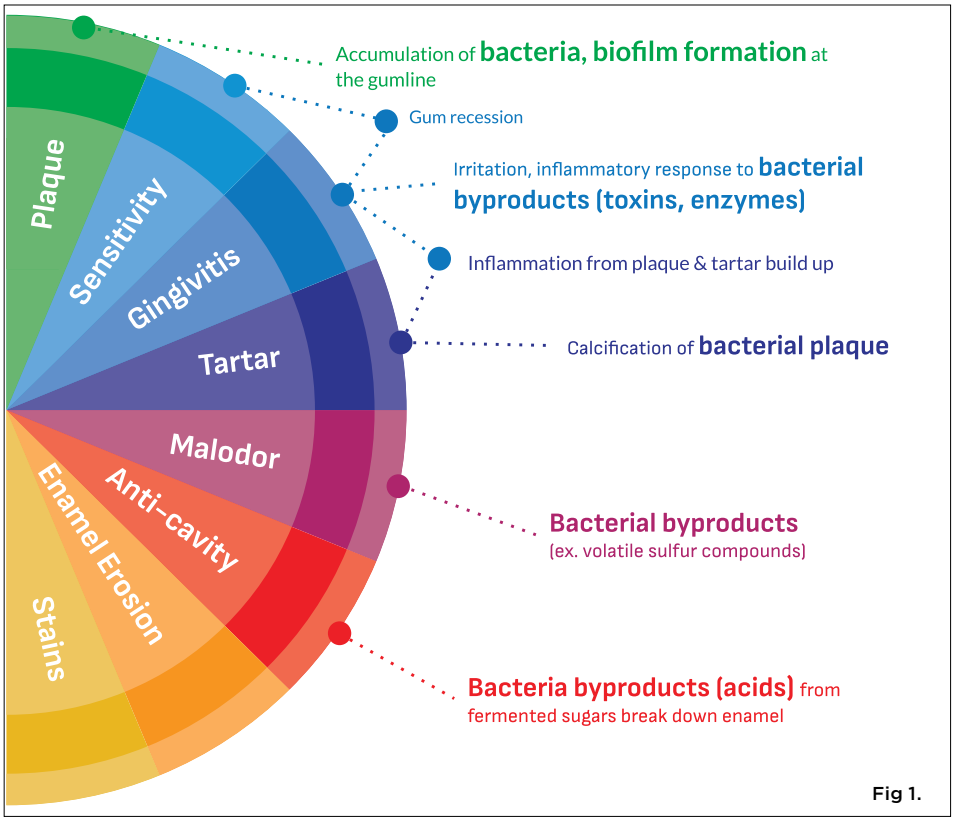

Fig 1. Oral bacteria can connect as a root cause of many common oral health issues.

Stannous Fluoride: A Powerful Partner With Challenging Applicability

Stannous fluoride (SnF_2) is a well-known active ingredient in dentistry offering multiple oral care benefits.[23-28] SnF_2 offers both hard tissue and soft tissue benefits due to its multiple modes of action. Sn^{2+} ions are known to interfere with bacterial metabolic function, slowing their growth and preventing bacterial acid production.[29,30] SnF_2 has also been shown to form mineral precipitates on dental hard tissues like dentin and enamel.[31,32] These precipitates can occlude exposed dentin tubules, which are a major cause of dentin hypersensitivity. As a result of these diverse modes of action, SnF_2 dentifrices have shown clinically significant reductions in dental plaque formation, gingivitis, malodor, and hypersensitivity pain in addition to prevention of caries and enamel erosion.[28,33,34]

While the multiple benefits provided by SnF_2 are a clear advantage to other fluoride sources such as sodium fluoride and sodium monofluorophosphate, which only offer caries protection, SnF_2 dentifrices are challenging to formulate. While relatively inert as a simple salt, SnF_2 dissociates into its constituent ions (F^- and Sn^{2+}) in aqueous environments that are common in typical dentifrice formulations.[29,35] Aqueous Sn^{2+} ions are sensitive to air, heat, and water presenting a critical obstacle. Moreover, a dichotomy exists as Sn^{2+} ions are inherently unstable at the optimal conditions for bioactive fluoride. Fluoride ion stability is best in high-water formulations at near neutral or slightly alkaline pH (pH 7–9).[36-38] Stannous salts can readily hydrolyze under aqueous conditions, especially above pH 4, resulting in precipitation from solution and/or subsequent oxidation to Sn^{4+}.[30,39-41]

Because clinical efficacy is dependent on the Sn^{2+} state of the ion, it is paramount to try to maintain this oxidation state throughout the lifetime of the product.[29,42-44] If not strategically designed, a SnF_2 dentifrice can run the risk of poor efficacy, surface enamel staining, or a perceivable metallic taste. Even the US Food and Drug Administration (FDA) monograph acknowledges this, stating, "The careful formulation of stannous fluoride dentifrices to prevent rapid oxidation and hydrolysis, and thereby inactivation, of stannous ions is critical for clinical effectiveness of these dentifrices."[40] A delicate balance must be achieved between maximizing the long-term chemical stability of the active ions in the toothpaste, their potent bioactivity in the mouth, and delivery in a consumer acceptability product form for continued and consistent use. Historically, several different strategies have been pursued in consumer dentifrices. While these mechanisms help address SnF_2 stability and bioactivity, they can impact user acceptability. Taste, mouth feel, and product look can be extremely different in a stabilized SnF_2 toothpaste compared to an ordinary sodium fluoride toothpaste, limiting universal adoption and compliance. Anhydrous formulations can be useful to prevent hydrolysis and subsequent oxidation to the Sn^{4+} species but may compromise consumer experience in mouth feel or taste.[29] These toothpastes may also exhibit poor standup on a brush and messy textures resulting from a lack of adequate viscosity-building agents that function in low-water formulations.[45] Some products use additional sources of stannous salts, such as stannous chloride, to compensate for Sn^{2+} ions oxidized or hydrolyzed in the formulation. However, this approach was shown to be inefficient, perpetuating a high level of inactive Sn^{4+} ions in the toothpaste.[29] This method (like anhydrous formulas) can also lead to high-cost manufacturing or ingredients, limiting affordable options for all users. Chelation with ligands such as pyrophosphate, hexametaphosphate, or organic compounds has proven effective at slowing oxidation, likely

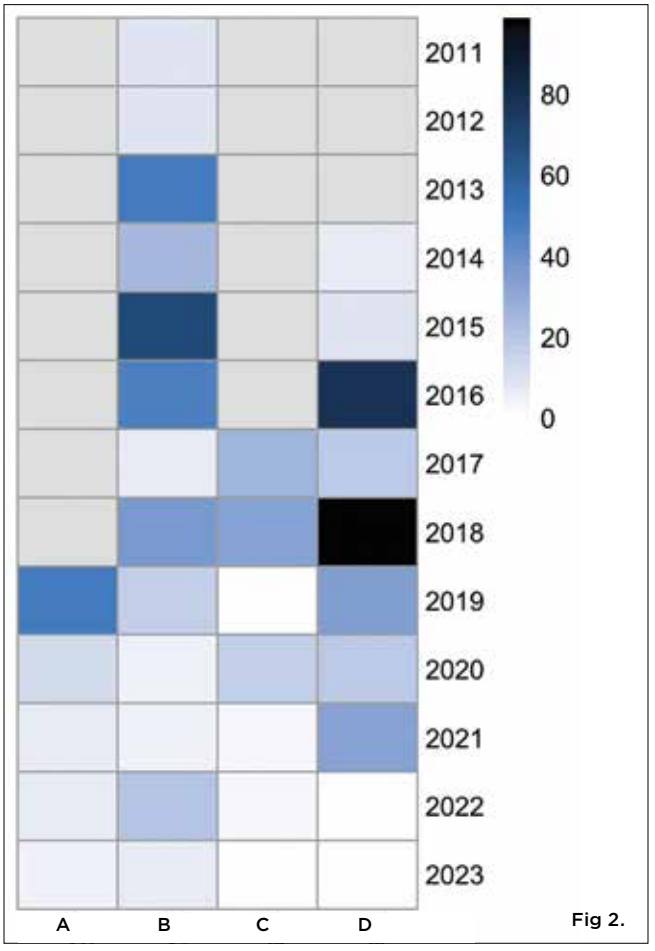

Fig 2. Heat map corresponding to the quantity of reported plausible adverse events (AEs) associated with stannous fluoride dentifrices (FDA AEs reporting system [FAERS] public dashboard).[48]
A = Colgate®, B = Crest®, C = Parodontax™, D = Sensodyne

through steric complexation mechanisms.[29,32,46] However, complexation mechanisms of Sn^{2+} must be carefully executed, as they may introduce solubility limitations or reduce their bioavailability. In rare cases, SnF_2 toothpastes with complex stabilization systems and limited water content have been implicated in the development of oral discomfort and other localized oral reactions such as oral mucosal exfoliation (Figure 2).[47,48] Given these collective complexities, the consumer products industry devotes significant resources to innovating new ways to optimize a balance of the chemical stability and bioactivity of Sn^{2+} ions in oral care products. However, these complex SnF_2 stabilization mechanisms usually have decreased formula flexibility, limiting major differentiation and innovation between products, such as building in new benefits and/or changes in foam, flavors, and product esthetics.

Colgate Total$^{SF®}$: A Breakthrough Stannous Toothpaste Stabilized by Phosphates

In 2019, Colgate Total$^{SF®}$ (Colgate-Palmolive Co., colgatepalmolive.com) was launched in the United States. This formula was comprised of SnF_2 stabilized in a single-phase formulation in the presence of phosphate sources such as tetrasodium pyrophosphate and zinc phosphate in an appropriate organic acid buffer system.[29] The toothpaste was formulated at near neutral pH in a high-water (>20%) formulation, helping maintain high levels of fluoride ion stability in the toothpaste matrix. X-ray absorption near edge spectroscopy (XANES) showed the ability of this chelation scheme to maintain higher levels of Sn^{2+} (both free ions and chelated ions) in this toothpaste formulation when compared to other commercially available SnF_2 toothpastes.[29] This system was also observed to be highly efficient when normalized to total stannous in the base. Considering the inclusion of only 0.454% SnF_2 in the toothpaste, the ratio of Sn^{2+} to inactive Sn^{4+} was 6.63; other commercially available SnF_2 toothpastes (including those supplemented with additional stannous salts) had significant levels of inactive Sn^{4+} (40% to 59% of the total stannous in the base) with ratios of less than 1.5.[29] Correlation between the Sn^{2+} and Sn^{4+} ratios in each product and antibacterial efficacy was observed in vitro.[29] Improved stain prevention was also observed with this toothpaste in vitro in assay comparisons to other SnF_2 commercial products highlighting the importance of oxidative stability imparted by the formulation scheme.[45] Clinical efficacy of this toothpaste has been demonstrated in multiple studies across a broad spectrum of oral health conditions, including reductions in plaque bacteria, gingivitis, and hypersensitivity pain.[31,49,50] The mode of action of the formulation has been examined in clinical studies from bacterial as well as the innate functions of the mouth.[51,52] Specific bacteria, bacterial gene pathways, and oral inflammatory biomarkers influenced by stannous have been identified, concomitantly resulting in healthy biofilm and reduced gingival inflammation.

Next Generation SnF_2: Stannous Stabilized by Nitrate and Phosphates

Recently, researchers discovered that a proprietary combination of phosphates and nitrate ions, two common ingredients in oral care, can help both solubilize and stabilize Sn^{2+} ions in a revolutionary manner.[53] These ingredients appear to work synergistically. Pyrophosphate-chelated Sn^{2+} ions maintain this chemistry as a water-soluble, bioactive form at near neutral pH. Simultaneously, nitrate appears to help block the chemical reaction pathways that lead to Sn^{4+} conversion, even under conditions known to promote oxidation, including heat, high-water environments, and dissolved oxygen gas. In simple aqueous solutions, this Sn^{2+} active engine showed almost nine times more Sn^{2+} (nearly 90% of starting amount) remaining after 2 weeks at 60°C in comparison to SnF_2 alone (<10% of starting amount remaining).[53] This system was also shown in vitro to significantly suppress the growth of known oral disease–causing bacteria (*Streptococcus mutans* and *Porphyromonas gingivalis*) and reduce production of the pro-inflammatory cytokine interleukin (IL)-8 in *P gingivalis* lipopolysaccharide–challenged cells.[53]

Performance testing, including clinical studies, of toothpastes formulated with 0.454% SnF_2 stabilized by nitrate and phosphates have shown impressive results. Application of this engine in various toothpaste backbones has demonstrated the safety, versatility,


Fig 3.


Fig 4.


Fig 5.


Fig 6.

Fig 3 through Fig 6. A wide variety of esthetics and user experiences (eg, foaming profiles, flavors, cooling agent intensity) can be developed, enabled by the unique advantages of the novel stannous fluoride stabilized by nitrate and phosphates.

and high quality associated with this approach. Many of these results are summarized in this special issue, including:

- significant and clinically relevant reductions of bacteria in saliva and on multiple oral surfaces (including teeth, tongue, cheeks, and gums) 12 hours post-brushing after 4 weeks of continuous use[54]
- clinical results showing superior reduction of plaque after 3 months of use; powerful gum care with 100% of patients in a clinical study showing improvement in gingival index over 6 months of continuous use[55]
- significant and clinically relevant reductions in hypersensitivity pain after 1 day (two times brushing) in comparison to a 5% potassium nitrate desensitizing toothpaste[56]
- significant reductions in oral malodor, with 85.7% of patients achieving organoleptic scores corresponding to pleasant breath overnight 12 hours post-brushing after 3 weeks of continued use.[57]

With this active engine, the manufacturer is also able to formulate against key consumer needs associated with lifestyle and environment, including clinically proven tooth stain reduction measured after 3 and 6 weeks of product use.[58]

Finally, this active engine enables an improved pathway to incorporate new benefits and user experiences in a SnF_2 toothpaste. Streamlined and discrete, this technology needs only two ingredients at low levels to ensure SnF_2 stability in a high-water-content toothpaste at near neutral pH, leading to less complexity in manufacturing, fewer flavor restrictions required to mask unfavorable metallic tastes, reduced risk of teeth staining, and decreased toothpaste discoloration. Furthermore, the simple phosphate chelation mechanism used in this engine not only helps limit interactions with a wide variety of formula excipients but also maintains the bioactivity of this active engine upon brushing. The unique combination provides a new opportunity for flexible formulation of a diverse portfolio of efficacious SnF_2-based formulas. In addition to innovation through new benefits and functional ingredients, a wider variety of experiences (foaming profiles, flavors, cooling agents, esthetics) can now be developed to engage and adapt to the diverse preferences of a global population (Figure 3 through Figure 6). In consumer studies, users rated the new toothpaste higher in flavor and foaming attributes (in comparison to the original Colgate Total^{SF} toothpaste). They also saw the new product as better matched with health-based attributes like "provides long lasting protection for my mouth" and "allows me to be proactive about my oral health."[57]

This novel SnF_2 bioactive engine enables a new, expanded people-centered approach focused on the toothbrushing experience while maintaining multi-benefit efficacy against plaque, gingivitis, cavities, calculus, hypersensitivity, enamel erosion, oral malodor, and tooth staining.

Evolving to a New Era

Colgate's bioactive SnF_2 engine leveraging nitrate and phosphates stabilization technology offers a novel, streamlined, efficacious approach to SnF_2 stability and bioavailability, with distinct advantages, including more efficient manufacturing and enhanced formulation flexibility for broader versatility in flavors, cooling agents, esthetics, and foaming profiles. This novel SnF_2 bioactive engine enables a new, expanded people-centered approach focused on the toothbrushing experience while maintaining multi-benefit efficacy against plaque, gingivitis, cavities, calculus, hypersensitivity, enamel erosion, oral malodor, and tooth staining.

Prevention strategies work most effectively with compliance.[59,60] This engine has also created a unique, new opportunity given its advantages in flavoring, complexity reductions, and resistance to excipient ingredient interactions. Even in a therapeutic toothpaste, the right flavor, foam, color, mouth feel, texture, and cosmetic benefits help to drive compliance. However, different users can have vastly different perceptions and preferences that manifest as reasons to believe or not believe in a product's performance and continued use. The development, customization, and curation of unique experience profiles befitting different groups better promotes consistent use, enabling as many different groups of users as possible to experience the scientific and clinical benefits of a SnF_2 toothpaste.

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DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Antibacterial Effects of a Novel Stannous Fluoride Toothpaste Stabilized With Nitrate and Phosphates (SNaP): In Vitro Study and Randomized Controlled Trial

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Abstract: *Background:* Stannous fluoride has long been an effective antibacterial, anticaries, antisensitivity, and antigingivitis addition to toothpaste formulas. However, in the past its chemical properties in aqueous solution have made it difficult to stabilize with desirable results. The recent development of a novel formulation of 0.454% stannous fluoride stabilized with nitrate and phosphates (SNaP) has resulted in prolonged therapeutic effect without compromising product experience and esthetics. *Methods:* Dentifrice antibacterial performance in vitro was determined through bacterial bioenergetics measured via rate of oxygen consumption and extracellular acidification in real-time comparing the SNaP toothpaste, a stannous fluoride positive control toothpaste, a non-antibacterial negative control toothpaste, and no treatment. Also, a single-center, randomized, controlled, double-blinded, clinical investigation of 98 subjects was performed to analyze dentifrice antibacterial performance in vivo following twice daily treatment with SNaP toothpaste (n = 48) and non-antibacterial control toothpaste (n = 50). Oral microenvironments, including plaque, tongue, cheek, gum, and saliva, of study participants 12 hours post-brushing were analyzed for bacterial load at baseline, 2 weeks, and 4 weeks. *Results:* In vitro treatment of biofilms with SNaP toothpaste resulted in significant suppression of bacterial respiration and glycolysis compared to a positive control, negative control, and no treatment. In the clinical trial, treatment with SNaP toothpaste showed significantly lower bacterial load in all oral microenvironments 12 hours post-brushing after 2 weeks (all: $P < .01$) and 4 weeks (all: $P < .05$) compared to non-antibacterial negative control toothpaste. Compared to baseline, SNaP toothpaste significantly reduced bacteria from tongue ($P = .007$) and saliva ($P < .001$) at week 2, and from all microenvironments by week 4 (all: $P \leq .001$). *Conclusions:* SNaP toothpaste provided significantly greater and more sustained antibacterial effects than other tested toothpastes. Stannous fluoride, when stabilized in the SNaP formulation, effectively inhibited bacterial respiration and glycolysis in saliva-derived in vitro biofilms. The specific stabilization strategy used in SNaP toothpaste is critical for the antibacterial performance of stannous fluoride, as this formulation was more effective at reducing bacterial metabolic activity than a toothpaste containing the same amount of stannous fluoride stabilized with gluconate. The clinical study supports the in vitro findings by showing that the regular use of SNaP toothpaste leads to a significant and prolonged reduction in viable bacterial counts of five oral microenvironments. *Practical Implications*: The highly stabilized stannous ion in SNaP toothpaste confers potent, sustained antibacterial activity that can contribute to improved oral hygiene and potentially reduce the risk of tooth decay, early gum disease, calculus, and halitosis, which have been linked to oral bacteria.

Humans are host to vast communities of microbiota that, when maintained in balance, are critical to human immunological, metabolic, and physiological function. Multiple distinct microbial habitats exist just within the mouth.[1] These oral microenvironments, such as the teeth, tongue, cheek, gum, and saliva, support the growth of highly heterogeneous and significantly different bacterial assemblies.[2] Bacteria in community naturally form complex networks, called biofilms, which attach to these oral surfaces; but the combination of the nutrient-rich environment of the mouth and nature of modern-day dietary consumption can result in the accumulation of oral biofilms into dental plaques.[3] Plaque control plays an integral role in preventing microbial imbalances that lead to dental caries, gingivitis, and periodontitis.[4] Thus, toothbrushing with fluoridated toothpaste and interdental prophylaxis are recommended to fight caries and reduce plaque accumulation. However, 42.2% of adults in the United States over 30 years old have mild, moderate, or severe periodontitis.[5] Evidence suggests that the large percentage of adults with poor oral health is the result of poor technique or irregularity of brushing and the type of dentifrice used, which could be improved by the inclusion of a toothpaste with antimicrobial properties.[6]

Stannous fluoride has long set a historical precedent as a highly effective antibacterial, anticaries, and antigingivitis addition to toothpaste formulas. The antibacterial performance of SnF_2 outperforms other fluoride-based therapeutics and is dependent on maintaining Sn^{2+} ions, which interfere with bacterial metabolic function.

Stannous fluoride (SnF_2) has long set a historical precedent as a highly effective antibacterial, anticaries, and antigingivitis addition to toothpaste formulas.[7-9] The antibacterial performance of SnF_2 outperforms other fluoride-based therapeutics and is dependent on maintaining Sn^{2+} ions, which interfere with bacterial metabolic function, slowing growth, reducing cellular respiration, and preventing production of bacterial acid through glycolysis.[8,10] However, in aqueous conditions SnF_2 can readily hydrolyze to form therapeutically inactive Sn^{4+} ions.[10-13] Previous approaches to improve on Sn^{2+} ion stability have included the removal of water to prevent hydrolysis, which is costly, presents processing difficulties, and compromises product desirability, and the addition of more Sn^{2+} salts, which compromises taste and can increase tooth staining.[14] An additional approach involves the use of complexation agents, such as gluconate, lactate, and polyphosphates, to chelate stannous. Clinical efficacy of SnF_2 toothpastes relies on the prolonged stability of the 2+ oxidation state of stannous.

The recent development of a proprietary SnF_2 formula combines the properties of phosphates and nitrate ions to solubilize and stabilize Sn^{2+} ions, prolonging the therapeutic efficacy of both F^- and Sn^{2+} without compromising flavor, mouth feel, or whitening capabilities.[14-16] In vitro studies reveal that this formulation significantly suppresses the growth of *Streptococcus mutans* and *Porphyromonas gingivalis*, while approximately 90% of Sn^{2+} ions remain in solution after 2 weeks.[14]

The objective of the study herein was twofold. The first portion was an in vitro study measuring the prolonged antimicrobial impact of toothpaste containing 0.454% stannous fluoride stabilized with nitrate and phosphates (SNaP) on cultured salivary biofilms compared to a commercially available SnF_2 toothpaste formulation and a non-antibacterial, non-stannous, fluoride toothpaste. The second part was a clinical study to evaluate the antibacterial effects of toothpaste containing SNaP compared to a non-antibacterial negative control sodium monofluorophosphate toothpaste in vivo.

Materials and Methods

In Vitro Biofilm Investigation

Dentifrice Treatment: The test treatment was toothpaste containing 0.454% stannous fluoride stabilized with nitrate and phosphates (SNaP). The negative control in this portion of the study was a non-antibacterial toothpaste containing 0.24% sodium fluoride (NaF) and 5% potassium nitrate (GlaxoSmithKline Co., gsk.com). The positive control treatment was toothpaste containing 0.454% stannous fluoride stabilized with sodium gluconate (SnF_2 + SG) (Procter & Gamble, pg.com).

Biofilm Culture: Biofilms derived from saliva of healthy volunteers were cultured vertically on hydroxyapatite discs for approximately 48 hours at 37°C, 5% carbon dioxide aerobic conditions in McBain medium containing 5 µg/ml hemin and 1 µg/ml vitamin K.[17] The media were replaced twice daily at approximately 12-hour intervals. The biofilm from one disc was transferred to a 24-well plate containing 1 ml of McBain medium. The biofilm was further dispersed by vigorous pipetting and transferred to an adjacent well. Toothpaste treatments were applied to 15 µl of the dispersed biofilm suspension, which contained approximately 10^6 cells per suspension.

Bacterial OCR and ECAR Measurements: Bacterial metabolic function[18,19] was measured using a Seahorse XFe24 cell analyzer (Agilent, agilent.com). Bioenergetics following treatment with the toothpastes were quantified in real time by measuring oxygen consumption rate (OCR; pmol/min) and extracellular acidification rate (ECAR; mpH/min). Briefly, bacteria were seeded and immobilized in microplates using Cell-Tak™ (Corning, ecatalog.corning.com), and bacterial metabolism in the presence or absence of treatments was measured for up to 200 minutes. Toothpaste slurries were prepared by mixing one part of toothpaste with eight parts of water, after which the slurry was spun down to remove solid material, and 10 µl of toothpaste supernatant was used in each experimental well. The experiments were carried out in

375 µl of McBain media at 37°C with intermittent shaking. The area under the curve was calculated for each treatment using GraphPad-Prism version 9.0 for Windows (GraphPad Software, graphpad.com). Data are representative of four independent experiments performed at least in triplicates. A two-sided t-test was used to assess statistical significance.

Clinical Investigation

Dentifrice Treatment: The test treatment was SNaP toothpaste containing 0.454% stannous fluoride. The negative control treatment in the clinical portion of the study was a non-antibacterial toothpaste containing 0.76% sodium monofluorophosphate (MFP) (Colgate-Palmolive Co., colgatepalmolive.com).

Study Design and Participants: This double-blind, single-center, two-arm, parallel, randomized, controlled clinical investigation (NCT06353165) was approved by the Institutional Review Board of Mahidol University. One-hundred healthy female and male adults (50 participants per group) between the ages of 18 and 70 years old were recruited March 1–3, 2023, from the greater Bangkok, Thailand, area. The study took place between March 8, 2023, and April 12, 2023. The number of participants needed for this study was calculated as previously described.[20,21] Qualifying participants were randomized into two treatment groups. Randomization was performed using the random number calculator of the GraphPad QuickCalcs website: graphpad.com/quickcalcs (accessed March 2023). The test group received non-identifiable SNaP toothpaste, and the negative control group received MFP toothpaste. Original toothpaste tubes were covered with coded white labels.

Treatment with the novel SnF_2 dentifrice formula resulted in significant and clinically relevant suppression of bacterial metabolism and growth across in vitro and in vivo studies. Despite equal amounts of the active ingredient SnF_2, SNaP consistently outperformed the comparator toothpaste in a prolonged manner in vitro.

Inclusion and Exclusion Criteria: Inclusion criteria were as follows: a minimum of 20 natural teeth with facial and lingual scorable surfaces; a baseline whole-mouth score of dental plaque of 1.5 or more[22,23] and gingivitis index of 1.0 or more[24,25]; no allergies to oral hygiene formulations; a willingness to comply with all study procedures and clinical examination schedules. Exclusion criteria were as follows: a history of active or severe periodontal disease and loose teeth; gross dental caries; severe generalized abrasion of dental cervix and/or enamel; large tooth fracture or temporary restoration (based on visual examinations); fixed or removable orthodontic appliance or removable partial dentures; dental prophylaxis or treatments within the preceding month or during the study period; use of phenolic flavored products (eg, mint flavored candies and chewing gum) during the study period; difficulty complying with study procedures and examinations (eg, excessive gagging during oral assessment or inability to refrain from oral hygiene for 12 hours prior to scheduled visit); medical treatments including antibiotic, anti-inflammatory, or anticoagulant therapy during the preceding month or during the study period; history of medical conditions requiring prophylactic antibiotic treatment prior to invasive dental procedures; history of significant adverse effects following use of oral hygiene products such as toothpastes and mouthrinses; allergy to personal care/consumer products or their ingredients; history of alcoholism or recreational drug use (including habit-forming products), diabetes, hepatic or renal disease, inflammatory conditions, or serious transmittable diseases (eg, HIV); participation in another clinical study or test panel involving oral hygiene formulations within the preceding month; having a scheduled medical procedure during the study period; self-reported pregnancy or lactation during the study period.

Study Protocol: Participants were given a soft-bristled toothbrush and instructed to brush twice daily for 2 minutes using a full ribbon of toothpaste and to refrain from using other oral hygiene products during the study. Non-antibacterial negative control toothpaste, MFP, was provided for a 7-day washout period.

Twelve hours after the last day of the washout period, participants returned to the study site for the baseline evaluation (day 0), where investigators examined the oral cavity and asked safety questions. Oral microenvironment samples were collected in the form of oral rinse saliva, supragingival plaque, tongue scrapings, buccal mucosa scrapings, and gum scrapings. Participants received prelabeled anonymized toothpastes containing either SNaP or MFP. Participants returned for examination, questioning, and sample collection at 14 days + 12 hours for the week 2 (day 15) and 28 days + 12 hours for the week 4 (day 29) timepoints.

Sample Collection and Microbial Procedures: Saliva was collected by providing participants with 15 ml of sterile saline to rinse their mouths for 30 seconds and expectorate into a prelabeled sterile tube. Supragingival plaque was randomly collected from buccal surfaces of the upper right or left quadrant (teeth Nos. 2 through 8 or teeth Nos. 9 through 15) using a sterile Columbia 13/14 scaler, pooled, and placed in a tube containing 1 ml of sterile phosphate buffered saline (PBS). Tongue, cheek, and gum surface samples were collected using the edge of a sterile wooden disposable tongue depressor and comprised five scrapes per site from a defined area randomly chosen. The inside of the right or left cheek or an entire arch of the upper or lower jaw was scraped. Each tongue depressor was then placed into a tube containing 3 ml of sterile PBS. Oral microenvironment samples were vortexed for 30 seconds to shake loose the biofilms and sonicated for 30 seconds before serial dilution in PBS and plating on agar enriched with 5% sheep blood as previously described.[26]

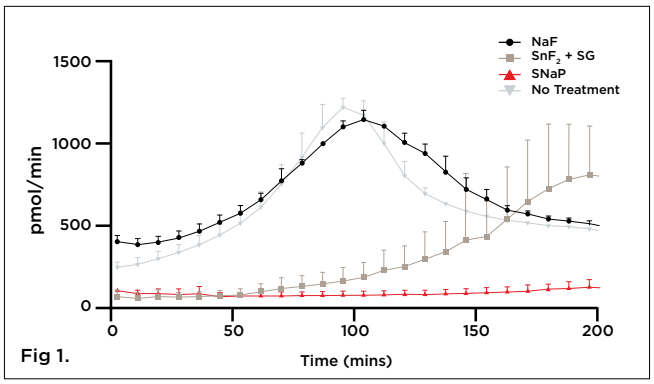

Fig 1.

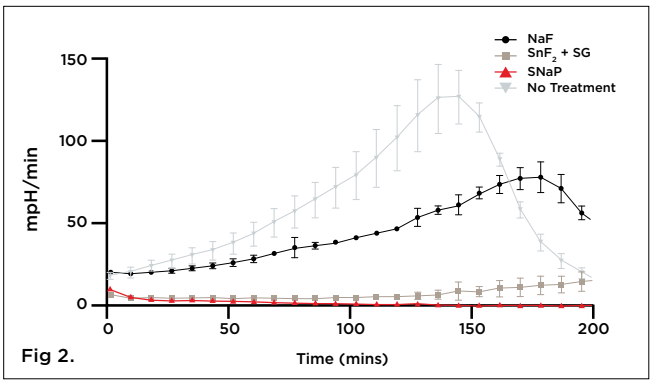

Fig 2.

Fig 1 and Fig 2. Bacterial bioenergetics analysis via a cell analyzer. Bacterial metabolism derived from salivary biofilm was measured over 200 minutes for: (Fig 1) real-time oxygen consumption rate (OCR) and (Fig 2) extracellular acidification rate (ECAR).

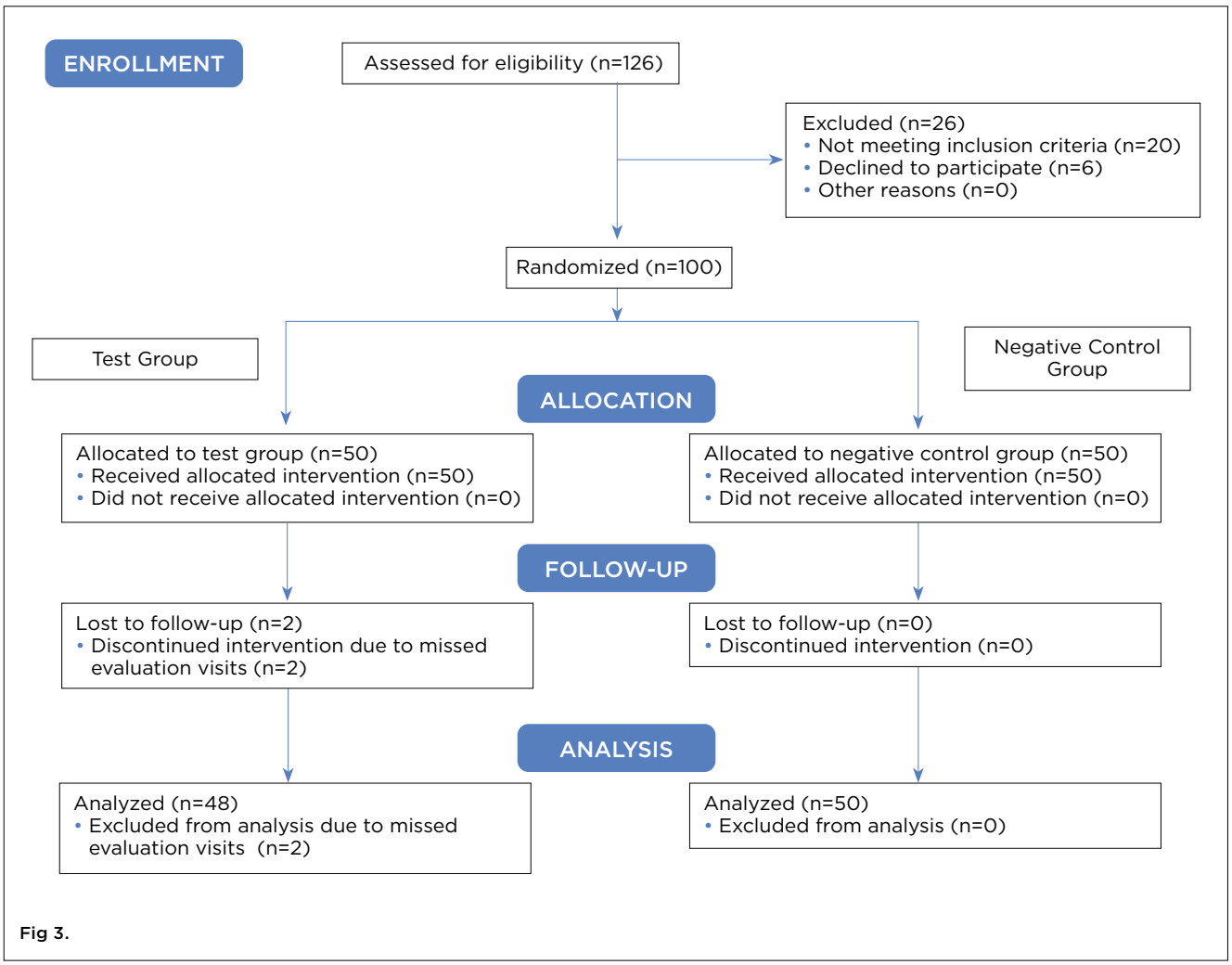

Fig 3.

Fig 3. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study population for the 4-week antibacterial clinical study.

Statistical Analyses

The gender and age compositions of the two treatment groups were compared using chi-squared analysis and analysis of variance (ANOVA), respectively. Raw data were logarithmically transformed (base 10) prior to statistical analysis. Baseline values between the two treatment groups were compared using ANOVA. Within-treatment baseline versus week 2 and week 4 comparisons were performed using paired t-tests. Between-treatment comparisons of baseline-adjusted week 2 and week 4 oral sample values were performed using analysis of covariance (ANCOVA) with

TABLE 1

In Vitro Rate Comparison in Bacterial Metabolic Function Post-Treatment

TREATMENT	AREA UNDER THE CURVE OCR (PMOL/MIN)	AREA UNDER THE CURVE ECAR (MPH/MIN)
No treatment	46946 ± 1985	6587 ± 309
Non-antibacterial fluoride	40155 ± 1260	4613 ± 110
Stannous fluoride + SG	19628 ± 3348	899 ± 86.3
Stannous fluoride (SNaP)	863.8 ± 51.3*	308 ± 8.32*

Area under the curve for oxygen consumption rate (OCR) was calculated after the first 10 cycles, ie, after 80 minutes and up to 160 minutes post–toothpaste treatment. Area under the curve for extracellular acidification rate (ECAR) was calculated after the first 10 cycles, ie, after 80 minutes post–toothpaste treatment.
*P < .05 compared to stannous fluoride + SG toothpaste, non-antibacterial fluoride toothpaste, and no treatment
SG = sodium gluconate, SNaP = stabilized with nitrate and phosphates

TABLE 2

Study Group Characteristics

TREATMENT GROUP	NUMBER OF PARTICIPANTS (FEMALE)	MEAN AGE, YEARS (SD)	AGE RANGE, YEARS
Test	48 (21)	39.65 (9.52)	24–56
Negative Control	50 (24)	39.28 (8.47)	24–53

No statistically significant difference was indicated between the two groups with respect to gender and age.
SD = standard deviation

TABLE 3

Baseline Bacterial Load From Oral Microenvironments

SOURCE	TREATMENT GROUP	BASELINE MEAN (SD)	*P* VALUE
Plaque	Test	7.47 (0.23)	.038
	Negative Control	7.33 (3.38)	
Tongue	Test	6.86 (0.29)	.364
	Negative Control	6.82 (0.21)	
Cheek	Test	5.85 (0.20)	.565
	Negative Control	5.89 (0.34)	
Gum	Test	6.13 (0.28)	.231
	Negative Control	6.20 (0.27)	
Saliva	Test	7.19 (0.21)	.037
	Negative Control	7.08 (0.27)	

Baseline mean reported as log10 CFU/mL. Statistics were conducted with an independent t-test.
SD = standard deviation

baseline value as covariate. All statistical tests of hypotheses were two-sided and used a significance level of α = 0.05. Minitab version 18.1 (Minitab, minitab.com) was used to perform the analyses. The percent change in bacterial counts was calculated by using (1-10^ΔLog CFU) x 100%.

Results

In Vitro Biofilm Investigation

To compare the antibacterial performance of the novel SNaP formulation to other commercially available dentifrices in vitro, biofilm cultures derived from saliva of healthy volunteers were processed with the SNaP test toothpaste, a market brand stannous toothpaste (SnF_2 + SG), a non-antibacterial, non-SnF_2 negative control toothpaste (NaF), and no treatment. Antibacterial performance was measured by monitoring bacterial metabolic function for bacterial respiration via OCR and glycolysis via ECAR. Over a 200-minute observation period, SNaP toothpaste treatment exhibited notable suppression of OCR and ECAR compared to both the market brand stannous formula and negative control (Figure 1 and Figure 2). SnF_2 + SG showed OCR suppression for the first 50 minutes but bacterial respiration proceeded to increase over time for the remaining trial, while SNaP toothpaste suppressed OCR for the entire 200 minutes (Figure 1). Glycolytic activity was suppressed by both SnF_2 + SG and SNaP toothpaste treatments compared to the negative control; however, SNaP continued to keep ECAR at near zero levels throughout the entire experiment (Figure 2).

To further assess the significance of SNaP toothpaste suppression of bacterial respiration and glycolysis, area under the curve was measured and statistical significance was analyzed (Table 1). Both the SNaP and SnF_2 + SG toothpaste treatments were significantly lower than NaF negative control and no treatment in OCR and ECAR (P < .05). SNaP and SnF_2 + SG treatments resulted in 97.8% and 51.1% less oxygen consumption than the NaF negative control treatment, respectively. Notably, the level of oxygen consumption following SNaP toothpaste treatment was 95.6% less than the SnF_2 + SG dentifrice (P < .05). SNaP and SnF_2 + SG treatments also resulted in a 93.3% and 80.5% lower rate of glycolysis than treatment with the NaF negative control, respectively. Again, the level of glycolysis following SNaP toothpaste treatment was 65.7% less than the SnF_2 + SG positive control (P < .05). There was no statistically significant difference between NaF negative control treatment and no treatment in both OCR and ECAR.

Clinical Investigation

Study Design: One-hundred participants were recruited and 50 were randomly allocated into either an antibacterial test group or

TABLE 4

Within-Treatment and Between-Treatment Mean Bacterial Load at Week 2 for Oral Microenvironments 12 Hours Post-Brushing

SOURCE	TREATMENT GROUP	ADJUSTED MEAN (SE)	WITHIN-TREATMENT		BETWEEN-TREATMENT	
			% Change	*P* Value	% Difference	*P* Value
Plaque	Test	7.42 (0.00)	10.9	.142	14.9	< .001
	Negative Control	7.49 (0.00)	-44.5	.004		
Tongue	Test	6.75 (0.00)	22.4	.007	14.9	< .001
	Negative Control	6.82 (0.00)	0.0	.833		
Cheek	Test	5.81 (0.02)	8.8	.195	24.1	< .001
	Negative Control	5.93 (0.02)	-9.6	.380		
Gum	Test	6.14 (0.02)	-2.3	.859	16.8	.003
	Negative Control	6.22 (0.02)	-4.7	.569		
Saliva	Test	7.04 (0.00)	29.2	< .001	18.7	< .001
	Negative Control	7.13 (0.00)	-12.2	.203		

Baseline-adjusted mean reported as log10 CFU/mL. Within-treatment *P* value calculated with paired t-test relative to baseline. Percent reduction exhibited by the 2-week mean relative to the baseline mean. A positive value indicates a reduction in bacteria (log10 CFU/mL) at the 2-week examination. Between-treatment *P* value calculated with ANCOVA of baseline-adjusted mean at week 2. Difference between the adjusted 2-week mean expressed as a percentage of the adjusted 2-week mean for the Negative Control group. A positive value indicates a greater reduction in bacteria (log10 CFU/mL) for the Test group relative to the Negative Control group.
SE = standard error

a non-antibacterial negative control group (Figure 3). No statistically significant difference was indicated between the two treatment groups with respect to gender (*P* = .673) and age (*P* = .841) (Table 2). Of the 100 study participants, 98 completed the study (Figure 3). Two participants from the test group did not attend all study visits for reasons unrelated to adverse events and were excluded from analysis.

The per protocol participants brushed twice daily for 2 minutes using a full ribbon of toothpaste and refrained from use of other oral hygiene products during the study. Following a 7-day washout period, oral microenvironment samples were collected at baseline, 2 weeks, and 4 weeks in the form of oral rinse saliva, supragingival plaque, tongue scrapings, buccal mucosa (cheek) scrapings, and gum scrapings. Statistical analyses were performed on oral microenvironment bacterial load log10 (CFU/mL) assessments.

Baseline Analysis: No significant difference was observed between the two treatment groups at baseline with respect to tongue (*P* = .364), cheek (*P* = .565), and gum (*P* = .231) bacterial load (Table 3). In saliva, the negative control group (7.08 ± 0.27 log10 CFU/mL) exhibited significantly (*P* = .037) less bacteria than the test group (7.19 ± 0.21 log10 CFU/mL). Plaque bacteria was also significantly (*P* = .038) lower in the negative control group (7.33 ± 3.38 log10 CFU/mL) than in the test group (7.47 ± 0.23 log10 CFU/mL).

Week 2 Analysis: By week 2, there was a significant difference between treatment group bacterial counts for all oral microenvironments (Table 4). The test group exhibited significantly lower bacterial loads than the negative control group for plaque (*P* < .001), tongue (*P* < .001), cheek (*P* < .001), gum (*P* = .003), and saliva (*P* < .001).

Compared to baseline measurements, the test group had a significant mean decrease in log10 CFU/mL bacterial load from tongue samples (*P* = .007) and saliva samples (*P* < .001). Meanwhile, the negative control group had a significant mean increase of log10 CFU/mL bacteria from plaque (*P* = .004). All other baseline-adjusted mean bacteria counts were not significantly different at 2 weeks compared to baseline (Table 4).

Week 4 Analysis: At week 4, there continued to be a significant difference between treatment group bacterial counts for all oral microenvironments (Table 5). The test group exhibited significantly lower bacterial loads than the negative control group for plaque (*P* < .001), tongue (*P* = .022), cheek (*P* < .001), gum (*P* < .001), and saliva (*P* = .001).

Four weeks after baseline measurements, the test group showed significant decrease in log10 CFU/mL bacteria from all oral microenvironments compared to baseline (Table 5). The negative control group only exhibited a significant decrease in log10 CFU/mL bacteria from tongue (*P* = .013) and saliva samples (*P* = .038).

Adverse Events: No staining of the participants' teeth was reported in either treatment group. No adverse events were noted during the examinations of the oral cavity at any visit, nor were any reported by the participants.

Discussion

Treatment with the novel SnF_2 dentifrice formula, SNaP, resulted in significant and clinically relevant suppression of bacterial metabolism and growth across in vitro and in vivo studies. Despite equal amounts of the active ingredient SnF_2, SNaP consistently outperformed comparator toothpaste, SnF_2 + SG, in a prolonged

TABLE 5

Within-Treatment and Between-Treatment Mean Bacterial Load at Week 4 for Oral Microenvironments 12 Hours Post-Brushing

SOURCE	TREATMENT GROUP	ADJUSTED MEAN (SE)	WITHIN-TREATMENT		BETWEEN-TREATMENT	
			% Change	*P* Value	% Difference	*P* Value
Plaque	Test	7.21 (0.03)	45.0	< .001	39.7	< .001
	Negative Control	7.43 (0.03)	-25.9	.122		
Tongue	Test	6.59 (0.04)	46.3	< .001	24.1	.022
	Negative Control	6.71 (0.03)	22.4	.013		
Cheek	Test	5.58 (0.03)	46.3	< .001	42.5	< .001
	Negative Control	5.82 (0.02)	14.9	.204		
Gum	Test	5.69 (0.03)	62.8	< .001	62.8	< .001
	Negative Control	6.12 (0.03)	16.8	.154		
Saliva	Test	6.83 (0.03)	56.3	< .001	30.8	.001
	Negative Control	6.99 (0.03)	20.6	.038		

Baseline-adjusted mean reported as log10 CFU/mL. Within-treatment *P* value calculated with paired t-test relative to baseline. Percent reduction exhibited by the 4-week mean relative to the baseline mean. A positive value indicates a reduction in bacteria (log10 CFU/mL) at the 4-week examination. Difference between the adjusted 4-week mean expressed as a percentage of the adjusted 4-week mean for the Negative Control group. A positive value indicates a greater reduction in bacteria (log10 CFU/mL) for the Test group relative to the Negative Control group. Between-treatment *P* value calculated with ANCOVA of baseline-adjusted means at week 4.
SE = standard error

manner in vitro. These results suggest that the nitrate/phosphates stannous stabilization approach used in SNaP toothpaste maintains bioavailable stannous to a greater extent than traditional stannous chelator systems like sodium gluconate. SNaP's antibacterial properties were also evident clinically, where it significantly reduced bacterial load in all oral microenvironments tested compared to commercially available non-antibacterial fluoride toothpaste 12 hours post-brushing after both 2 weeks and 4 weeks of continuous use.

SNaP toothpaste treatment of salivary biofilm cultures resulted in long-lasting and significant suppression of bacterial respiration and glycolytic activity in comparison to treatment with the same amount of SnF_2 stabilized with sodium gluconate. Studying bacterial respiration and glycolytic activity via OCR and ECAR is crucial for understanding how SNaP toothpaste impacts oral bacterial metabolism. By measuring OCR, researchers can determine how effectively the toothpaste inhibits the aerobic respiration of harmful oral bacteria, while ECAR measurements reveal its effects on glycolytic and fermentation processes. These insights are essential for evaluating the efficacy of a product in real-time, ensuring it effectively disrupts the metabolic activities of pathogens responsible for dental plaque and cavities. These results are supported by previous single-species bacterial inhibition studies that found that SNaP significantly inhibited the growth of common oral disease–associated bacteria, *S mutans* and *P gingivalis*, compared to SnF_2 without nitrate.[14] In aqueous solution, approximately 90% of Sn^{2+} ions remain in solution after 2 weeks when stabilized with phosphates and nitrate ions compared to <10% of Sn^{2+} that remain when SnF_2 is not stabilized with phosphates and/or nitrate ions.[14] This evidence indicates that the prolonged antibacterial effects of SNaP are due to the novel formula allowing for extended stability and bioavailability of Sn^{2+}.

The antibacterial properties of SNaP toothpaste seen in vitro were observed in vivo as well. After 4 weeks of continuous use, SNaP toothpaste significantly reduced bacterial count in saliva, supragingival plaque, tongue scrapings, buccal mucosa (cheek) scrapings, and gum scrapings compared to baseline levels in clinical trial groups. Notably, despite lower baseline levels of bacteria in plaque and saliva in the negative control group at baseline, bacteria from plaque and saliva increased to be higher than the SNaP toothpaste–treated test group by week 2. Use of SNaP toothpaste resulted in significantly lower amounts of bacteria 12 hours after brushing for all oral microenvironments tested compared to MFP toothpaste. These results are supported by evidence that 0.454% SnF_2 is able to significantly reduce biofilm formation, cell adhesion, and quorum sensing on tooth surfaces and oral devices compared to MFP.[27]

While previous studies have shown that brushing with a SnF_2 dentifrice reduces the buildup of dental calculus, plaque, gingivitis, tooth stain, and malodor,[28] the extended bioavailability observed from the SNaP formulation may allow for a greater long-term therapeutic effect. The sustained antibacterial impact on both hard- and soft-tissue surfaces may also prevent soft-tissue sites from acting as microbial reservoirs that reseed teeth with bacteria following prophylaxis.[6] The results of this can be seen in other SNaP clinical trials yielding prolonged malodor reduction along with significant reductions in plaque, gingivitis, and dentin hypersensitivity.[16,29,30]

Further research on the SNaP formula could elucidate the exact mechanisms of action driving its prolonged antibacterial efficacy. Recent research leveraging next-generation sequencing has shown that certain oral pathogens appear to be more impacted than others by stannous fluoride[27,31]; future studies could help uncover the comprehensive profiles of microbial communities affected by SNaP, offering insights into shifts in bacterial diversity and the relative abundance of oral microorganisms induced by this oral care ingredient. Identifying metabolic changes and biochemical pathways impacted by SNaP treatment and understanding how it modulates bacterial activity at the molecular level could help design formulation strategies to enhance its efficacy and provide long-term oral health outcomes.

Toothbrushing with fluoridated toothpaste and interdental prophylaxis are recommended to maintain oral health. However, periodontitis remains a clear issue for a large percentage of adults who may not have optimal brushing practices.[5,6] The incorporation of stable antibacterial ingredients like stannous fluoride in SNaP toothpaste is important to prevent bacterial-driven oral conditions.

Conclusion

SNaP toothpaste provides superior, sustained antibacterial effects that can help prevent the growth of harmful bacteria without any reported staining or other unpleasant consequences. The fact that these antibacterial effects were not limited to the enamel surfaces but also proven in four additional areas of the mouth supports the use of SNaP toothpaste to maintain whole-mouth health.

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DISCLOSURES

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DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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A 6-Month Randomized Controlled Trial to Measure the Efficacy of a Stannous Fluoride Toothpaste Stabilized With Nitrate and Phosphates (SNaP) on Dental Plaque and Gingivitis

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Abstract: *Background*: The objective of this randomized controlled trial was the comparison of a stannous fluoride (SnF_2) dentifrice stabilized with nitrate and phosphates (test) to a regular fluoride dentifrice (negative control) for the control of plaque and gingivitis over 6 months. *Methods*: A total of 80 adult participants were enrolled in this study that was conducted in Loma Linda, California. After randomization and blinding of study personnel and patients, enrolled participants were provided instructions for the use of their assigned dentifrice. At three visits (0, 3, and 6 months), various gingival and plaque indices were collected to determine the clinical efficacy of the SnF_2 stabilized dentifrice. These results were compared with the results of the negative control dentifrice. *Results*: A total of 77 participants completed the study. The test dentifrice demonstrated statistically significant reductions versus baseline in all plaque and gingivitis indices after 3 and 6 months of product use. The negative control dentifrice demonstrated significant reductions versus baseline in all plaque indices, but not gingivitis indices, after 3 months of product use and in all plaque and gingivitis indices after 6 months of product use, with the exception of the interproximal gingivitis index, which did not reach statistical significance. The test SnF_2 dentifrice showed statistically significant reductions in all plaque and gingivitis indices compared to baseline and to the negative control dentifrice after 3 months and 6 months of product use (all: $P < .001$). *Conclusions*: The results of this clinical trial showed statistically significantly improved clinical outcomes for reduction of gingival inflammation and improvement in plaque control over 6 months when using a new SnF_2 dentifrice stabilized with nitrate and phosphates as compared to the results from a regular fluoride dentifrice. *Practical Implications:* This newly formulated SnF_2 dentifrice may be of benefit to patients who need help controlling plaque biofilm and in reducing gingivitis, leading to an improvement in overall oral health.

Individuals with gum disease, or periodontal disease (PD), have an inflammatory condition that includes gingivitis and periodontitis. Gingivitis is the mildest form of PD,[1] and as the population ages, it is expected that more cases of gingivitis will occur. PD impacts approximately 40% of adults and 60% of those over 65 years of age,[2] although children can also be affected by PD.[3,4] By 2030, all members of the "baby boomer" generation will have turned 65 years of age and will represent one out of every five Americans.[5] This acceleration of the aging population is also seen globally with 10% of the global population older than 65 years of age in 2022, and this percentage is expected to increase to 16% by 2050 and 24% by 2100.[6]

Gum disease is a public health issue due to its high prevalence and the potential for significant health impacts. The burden of PD on healthcare systems is considered substantial, with high costs associated with treatment and lost productivity.[7] Furthermore, it is becoming increasingly evident that there is a connection between PD and systemic health with the following diseases implicated: cardiovascular disease, diabetes, gastrointestinal disease, Alzheimer's disease, respiratory infections, and others.[8,9] Therefore, it is important that effective strategies to prevent and treat gingivitis are available so that it does not develop into the more serious form of gum disease, periodontitis.

Ideally, toothbrushing would result in the complete removal of this oral biofilm. However, complete mechanical removal is not possible, and antibacterial agents such as SnF_2 can be incorporated into a dentifrice to improve the overall efficacy of biofilm control.

Stannous fluoride (SnF_2) dentifrices are known to provide consumers with multiple benefits, including assisting with the reduction of plaque bacteria,[10,11] reducing gingivitis,[12,13] aiding in relief of dental hypersensitivity,[14] and providing caries control.[15] The oral biofilm known as dental plaque is the cause of oral diseases such as caries, gingivitis, and periodontitis.[16] Ideally, toothbrushing would result in the complete removal of this oral biofilm. However, complete mechanical removal is not possible, and antibacterial agents such as SnF_2 can be incorporated into a dentifrice to improve the overall efficacy of biofilm control.[12,13]

Maintaining bioavailable stannous fluoride within dentifrice formulations has been a challenge because Sn^{2+} easily hydrolyzes and oxidizes and precipitates in water and oxygen-containing environments, decreasing its therapeutic efficacy. Recently, stannous fluoride was combined with nitrate and phosphates (SNaP), resulting in an improvement to both oxidative stability and solubility and, therefore, stannous bioavailability.[17]

This new dentifrice has undergone a series of laboratory and clinical tests to establish its efficacy and benefits to ensure that this new stabilization strategy has not compromised the antiplaque, antigingivitis, or other benefits offered by the dentifrice.[10,18-20] In this study, the test dentifrice, containing 0.454% stannous fluoride stabilized with nitrate and phosphates, was compared to a negative control dentifrice, which was a regular commercial dentifrice with 0.76% sodium monofluorophosphate, in a 6-month study to evaluate the performance of both dentifrices against dental plaque and gingivitis.

Material and Methods

Study Design

The sample size of 80 participants (40 per treatment group) was determined based on the standard deviation for the response measures of 0.58, a significance level of $\alpha = 0.05$, a 10% attrition rate, and an 80% level of power. This study was powered to detect a minimal statistically significant difference between study group means of 15%. The sample size calculation was based on historical data from a previous study.[21] This randomized, single-center, double-blind, parallel-group study included 80 participants. The dentifrices compared were the test dentifrice, SNaP, and the negative control dentifrice, a 0.76% sodium monofluorophosphate dentifrice. Both were manufactured by Colgate-Palmolive Co. (colgatepalmolive.com).

This was a double-blinded study with neither the participants nor study personnel involved in participant evaluation (including the dental examiner) aware of the identity of the products or which treatment a participant was receiving. The test products were distributed and accounted for by personnel who were not involved with study participant evaluations.

Ethics

The study was reviewed and approved by the Loma Linda University Health Institutional Review Board (Loma Linda, California). All participants signed an informed consent form.

Inclusion and Exclusion Criteria

Participants who were between the ages of 18 and 70 (inclusive) and were available for the duration of the 6-month study were eligible to participate. They had to be in good general health and have at least 20 uncrowned permanent natural teeth, excluding third molars. In addition, eligible participants were required to have an initial mean gingival index score of at least 1.0 as determined by the Löe-Silness gingival index scale and index[22] and an initial mean plaque index score of at least 1.5 as determined by the Turesky modification of the Quigley-Hein plaque index scale.[23]

Participants were excluded from the study if they had the presence of orthodontic bands, partial removable dentures, one or more tumors of the soft or hard oral cavity tissues, or any advanced PD (eg, purulent exudate, tooth mobility, or extensive loss of periodontal attachment or alveolar bone). Other exclusion criteria were the presence of five or more decayed carious lesions that required

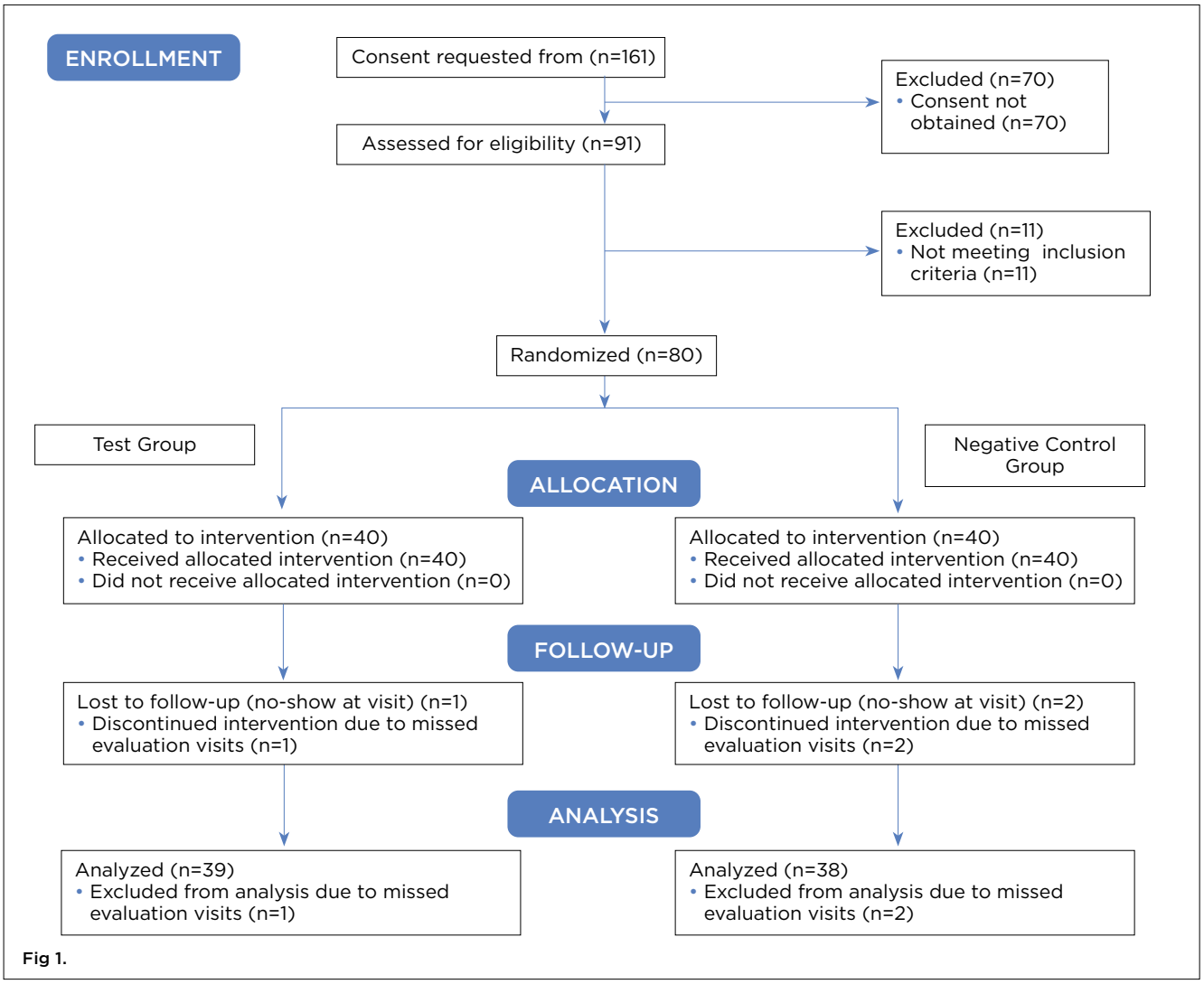

Fig 1.

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study population for the 6-month plaque and gingivitis clinical study.

immediate restorative treatment, a history of allergies to oral care/personal care consumer products or their ingredients, or the use of any prescription medicines that might interfere with the study outcome. Participants were excluded if they had a history of alcohol or drug abuse, were pregnant or lactating women, or had an existing medical condition that would prohibit them from eating or drinking for up to 4 hours. Within the 2 weeks prior to the start of the study, participants were not allowed to use of any antibiotics, and within 1 month prior to the baseline examinations, participants were not allowed to receive a dental prophylaxis. Participants could not participate in any other clinical study or test panel within 1 month before entry into the study.

Clinical Examination and Instructions

Qualifying participants were randomized to one of the two study treatments based on their initial gingivitis and plaque scores using a computer-generated list of random numbers. After randomization, participants were provided with their assigned dentifrice and a soft-bristled adult toothbrush for use at home. They were instructed to brush their teeth using the provided toothbrush for 2 minutes in the morning and in the evening (ie, twice a day), using approximately 1.5 grams of their assigned dentifrice. No instructions were provided to the subjects regarding brushing technique. The dentifrices were supplied in their original tubes but were overwrapped with a white adhesive label to conceal the product's identity. Label information on each tube consisted of the study treatment code, the instructions for at-home use, and safety information, including emergency contact information. The examiner obtained adverse event information through oral examination as well as interviews with the study participants during each study visit.

Scoring Procedures

Gingivitis Assessment: The degree of gingival inflammation was determined by dividing each tooth into six surfaces, and the

TABLE 1

Summary of Age and Gender of Subjects Who Completed the Clinical Study

TREATMENT GROUP	NUMBER OF SUBJECTS			AGE	
	Male	Female	Total	Mean (SD)	Range
Test	19	20	39	49.13 (12.89)	24–70
Negative Control	21	17	38	49.08 (13.68)	23–70
All Treatment Groups	40	37	77	49.10 (13.22)	22–70

SD = standard deviation

TABLE 2

Summary of Racial/Ethnicity Distribution of Study Participants Who Completed the Clinical Study

TREATMENT GROUP	RACE/ETHNICITY					
	White	African American (Black)	Hispanic/ Latino	Asian	Other	Total
Test	13 (33.3%)	1 (2.6%)	15 (38.5%)	10 (25.6%)	0 (0.0%)	39 (100%)
Negative Control	9 (23.7%)	3 (7.9%)	14 (36.8%)	10 (26.3%)	2 (5.3%)	38 (100%)

gingival index (GI) was scored as per the Löe-Silness gingival index.[22] Subject-wise whole-mouth scores were calculated by summing all scores for all sites and dividing by the total number of assessed sites.

Gingival Severity: Only those sites that had GI scores of 2 or 3 at baseline were included in these calculations. The gingivitis severity index (GS) was determined by counting those sites and dividing by the total number of assessed sites.

Gingival Interproximal: The gingivitis interproximal index (Gint) score was determined by counting the scores from the mesiofacial, distofacial, mesiolingual, and distolingual surfaces of each tooth and dividing the sum by the total number of assessed sites.

Dental Plaque Assessment: A red dye solution was used to disclose plaque, and plaque index score (PI) was determined using the Turesky modification of the Quigley-Hein index.[23] Subject-wise whole-mouth scores were calculated by summing all scores for all sites and dividing by the total number of assessed sites.

Plaque Severity: Only the distofacial, mesiolingual, and distolingual surfaces whose assigned PI scores were 3, 4, or 5 at baseline were included in these calculations. The plaque severity index (PS) was determined by counting those sites and dividing the sum by the total number of assessed sites.

Plaque Interproximal: The plaque interproximal index (Pint) score was determined by counting the scores calculated for each participant by adding the mesiofacial, distofacial, mesiolingual, and distolingual scores of each tooth and dividing the sum by the total number of assessed sites.

Qualifying participants were randomized to one of the two study treatments based on their initial gingivitis and plaque scores using a computer-generated list of random numbers. After randomization, participants were provided with their assigned dentifrice and a soft-bristled adult toothbrush for use at home.

Statistical Analysis

For age and sex, independent t-tests and chi-squared tests were conducted, respectively. Statistical analyses were performed separately for the gingivitis assessments and dental plaque assessments. Comparisons of the treatment groups with respect to baseline gingival index scores and plaque index scores were performed using an analysis of variance (ANOVA). Within-treatment comparisons of the baseline versus 3-month and 6-month gingival and plaque index scores were performed using paired t-tests. Comparisons of the treatment groups with respect to baseline-adjusted gingival and plaque scores at the 3-month and 6-month examinations were performed using analyses of covariance (ANCOVA). All statistical tests of hypotheses were two-sided and employed a level of significance of $\alpha = 0.05$.

TABLE 3

Mean Values at Baseline, 3 Months, and 6 Months for Each Treatment

INDEX	TREATMENT GROUP	BASELINE MEAN (SD)	3-MONTH MEAN (SD)	6-MONTH MEAN (SD)
GI	Test	1.35 (0.17)	1.10 (0.15)	0.86 (0.18)
	Negative Control	1.35 (0.19)	1.32 (0.24)	1.30 (0.24)
PI	Test	2.83 (0.42)	2.21 (0.41)	2.03 (0.34)
	Negative Control	2.87 (0.41)	2.66 (0.47)	2.60 (0.39)
GS	Test	0.36 (0.18)	0.14 (0.12)	0.03 (0.04)
	Negative Control	0.36 (0.20)	0.33 (0.23)	0.30 (0.23)
PS	Test	0.60 (0.13)	0.41 (0.15)	0.31 (0.15)
	Negative Control	0.61 (0.12)	0.53 (0.16)	0.51 (0.13)
Gint	Test	1.45 (0.21)	1.17 (0.17)	0.90 (0.21)
	Negative Control	1.44 (0.22)	1.41 (0.25)	1.39 (0.28)
Pint	Test	3.38 (0.48)	2.67 (0.54)	2.40 (0.46)
	Negative Control	3.46 (0.46)	3.22 (0.59)	3.11 (0.49)

GI = gingival index, Gint = gingival interproximal index, GS = gingival severity index, PI = plaque index, Pint = plaque interproximal index, PS = plaque severity index, SD = standard deviation

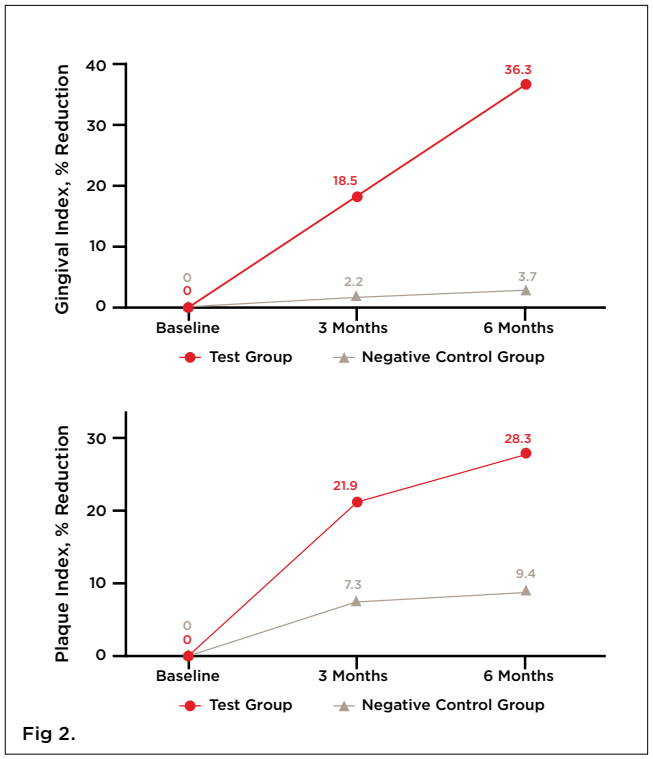

Fig 2. Percentage reduction relative to baseline for gingival index (top) and plaque index (bottom).

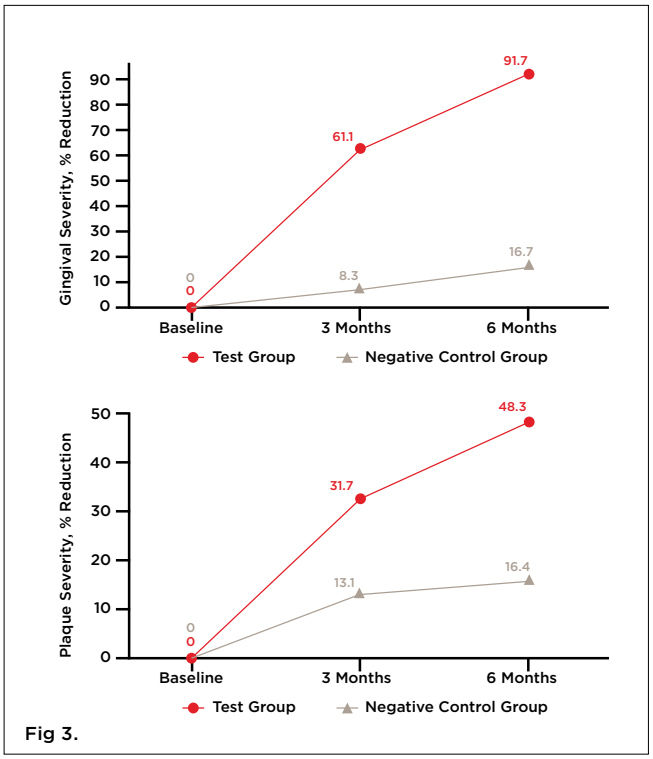

Fig 3. Percentage reduction relative to baseline for gingival severity index (top) and plaque severity index (bottom).

Results

A CONSORT flow diagram indicates the numbers of individuals involved at the various stages of the study (Figure 1). A total of 80 participants were randomized into the study with 40 participants in each treatment group. Three participants, one in the test group and two in the negative control group, did not complete the 6-month study and were not included in the analyses. They did not present themselves at each follow-up visit as required. The treatment groups did not differ significantly with respect to age (P = .726) or sex (P = .565), as shown in Table 1. A summary of the race/ethnicity of the study population is presented in Table 2.

TABLE 4

Statistical Parameters for Comparisons Made Within and Between Each Treatment Group at 3 Months*

INDEX	TREATMENT GROUP	BASELINE		WITHIN-TREATMENT			BETWEEN-TREATMENT	
		Mean (SD)	Adjusted Mean (SE)	Adjusted 95% CI	Percentage Reduction[1]	*P* Value[2]	Percentage Difference[3]	*P* Value[4]
GI	Test	1.35 (0.17)	1.11 (0.02)	1.07–1.15	18.5%	< .001	15.9%	< .001
	Negative Control	1.35 (0.19)	1.32 (0.02)	1.28–1.36	2.2%	.125		
PI	Test	2.83 (0.42)	2.23 (0.05)	2.13–2.33	21.9%	< .001	15.5%	< .001
	Negative Control	2.87 (0.41)	2.64 (0.06)	2.52–2.76	7.3%	.001		
GS	Test	0.36 (0.18)	0.14 (0.02)	0.10–0.18	61.1%	< .001	57.6%	< .001
	Negative Control	0.36 (0.20)	0.33 (0.02)	0.29–0.37	8.3%	.131		
PS	Test	0.60 (0.13)	0.41 (0.02)	0.37–0.45	31.7%	< .001	22.6%	< .001
	Negative Control	0.61 (0.12)	0.53 (0.02)	0.49–0.57	13.1%	.001		
Gint	Test	1.45 (0.21)	1.17 (0.03)	1.11–1.23	19.3%	< .001	17.0%	< .001
	Negative Control	1.44 (0.22)	1.41 (0.03)	1.35–1.47	2.1%	.200		
Pint	Test	3.38 (0.48)	2.70 (0.07)	2.56–2.84	21.0%	< .001	15.4%	< .001
	Negative Control	3.46 (0.46)	3.19 (0.07)	3.05–3.33	6.9%	.002		

*All indices were improved significantly from baseline except for the GI, GS, and Gint for the Negative Control group at 3 months.
1 Percent reduction exhibited by the 3-month mean relative to the baseline mean. A positive value indicates a reduction in index scores at the 3-month examination.
2 Significance of paired t-test comparing the baseline and 3-month examinations.
3 Difference between the adjusted 3-month mean expressed as a percentage of the adjusted 3-month mean for the Negative Control group. A positive value indicates a reduction in index scores for the test dentifrice relative to the negative control dentifrice.
4 Significance of ANCOVA comparison of baseline-adjusted 3-month means.
CI = confidence interval, GI = gingival index, Gint = gingival interproximal index, GS = gingival severity index, PI = plaque index, Pint = plaque interproximal index, PS = plaque severity index, SD = standard deviation, SE = standard error

Baseline

Table 3 shows the mean values for each treatment at baseline, 3 months, and 6 months for each gingival and plaque indice. For all gingival-based indices and all plaque-based indices there were no statistically significant differences between the two treatments at baseline.

3-Month Follow-up

After twice per day toothbrushing with the assigned dentifrice, participants' gingival and plaque indices were measured at the 3-month follow-up visit as they were at the baseline visit. All indices for the test dentifrice showed a statistically significant reduction from baseline (Table 4). For the negative control dentifrice, all plaque indices showed a statistically significant reduction from baseline, while none of the gingival indices did (Table 4). Figure 2 through Figure 4 show the percentage reductions over time for each of the treatments relative to baseline.

The test dentifrice provided statistically significant reductions in all plaque and gingivitis indices in comparison to the negative control dentifrice (Table 4). These reductions ranged from 15.4% for the plaque interproximal index to 57.6% for the gingival severity index.

6-Month Follow-up

After 6 months of twice per day toothbrushing with the assigned dentifrice, participants' gingival and plaque indices showed similar trends to that observed after 3 months. As shown in Table 5, the test dentifrice provided significant reductions in all gingival and plaque indices as compared to their baseline values. On the other hand, the negative control dentifrice provided significant reductions as compared to baseline for all gingival and plaque indices, except for the gingival interproximal index. Figure 2 through Figure 4 show the percentage reductions over time for each of the treatments relative to baseline. For all indices, the percentage reductions relative to baseline are larger for the test dentifrice as compared to the negative control dentifrice.

The test dentifrice provided statistically significant reductions in all plaque and gingivitis indices in comparison to the negative control dentifrice (Table 5). These reductions ranged from 21.2% for the plaque index to 90% for the gingival severity index. For all indices, the percentage difference between the two dentifrices increased as a function of time.

Additional Analyses

Furthermore, 97.4% (38 out of 39) of the study participants who

TABLE 5

Statistical Parameters for Comparisons Made Within and Between Each Treatment Group at 6 Months*

INDEX	TREATMENT GROUP	BASELINE		WITHIN-TREATMENT			BETWEEN-TREATMENT	
		Mean (SD)	Adjusted Mean (SE)	Adjusted 95% CI	Percentage Reduction[1]	*P* Value[2]	Percentage Difference[3]	*P* Value[4]
GI	Test	1.36 (0.17)	0.86 (0.03)	0.80–0.92	36.3%	< .001	33.8%	< .001
	Negative Control	1.45 (0.19)	1.30 (0.03)	1.24–1.36	3.7%	.017		
PI	Test	2.83 (0.42)	2.04 (0.06)	1.92–2.16	28.3%	< .001	21.2%	< .001
	Negative Control	2.87 (0.41)	2.59 (0.06)	2.47–2.71	9.4%	< .001		
GS	Test	0.36 (0.18)	0.03 (0.02)	-0.01–0.07	91.7%	< .001	90.0%	< .001
	Negative Control	0.36 (0.20)	0.30 (0.02)	0.26–0.34	16.7%	.011		
PS	Test	0.60 (0.13)	0.32 (0.02)	0.28–0.36	48.3%	< .001	37.3%	< .001
	Negative Control	0.61 (0.12)	0.51 (0.02)	0.47–0.55	16.4%	< .001		
Gint	Test	1.45 (0.21)	0.90 (0.04)	0.82–0.98	37.9%	< .001	35.3%	< .001
	Negative Control	1.44 (0.22)	1.39 (0.04)	1.31–1.47	3.5%	.081		
Pint	Test	3.38 (0.48)	2.41 (0.07)	2.27–2.55	29.0%	< .001	22.3%	< .001
	Negative Control	3.46 (0.46)	3.10 (0.07)	2.96–3.24	10.1%	< .001		

*All indices were improved significantly from baseline except for the Gint for the Negative Control group at 6 months.
1 Percent reduction exhibited by the 6-month mean relative to the baseline mean. A positive value indicates a reduction in index scores at the 6-month examination.
2 Significance of paired t-test comparing the baseline and 6-month examinations.
3 Difference between the adjusted 6-month mean expressed as a percentage of the adjusted 6-month mean for the Negative Control group. A positive value indicates a reduction in index scores for the test dentifrice relative to the negative control dentifrice.
4 Significance of ANCOVA comparison of baseline-adjusted 6-month means.
CI = confidence interval, GI = gingival index, Gint = gingival interproximal index, GS = gingival severity index, PI = plaque index, Pint = plaque interproximal index, PS = plaque severity index, SD = standard deviation, SE = standard error

brushed with the test dentifrice showed improvement in GI at 3 months and 100% (39 out of 39) showed improvement at 6 months. Conversely, only 63.2% (24 out of 38) of the participants who brushed with the negative control dentifrice showed improvement at 3 months and at 6 months.

Safety Results

Throughout the study, no adverse events on the oral hard or soft tissues were observed by the examiner or reported by the participants when questioned.

Discussion

Periodontitis and gingivitis have been associated with a negative influence on oral health–related quality of life (OHRQoL).[24] Recent work by Broomhead et al on 27 trial participants with PD (15 with gingivitis and 12 with periodontitis) determined that gingivitis impacted the participants' overall quality of life.[25] In particular, these individuals reported changing toothbrushes or their toothbrushing routines/techniques and avoiding chewy foods to prevent gum-related symptoms like bleeding, irritation, discomfort, and gum recession. Study participants switched to electric toothbrushes, which were perceived as more effective at tackling symptoms versus manual toothbrushes, and occasionally switched to softer toothbrushes to ease stress on the gums. Both groups also reported changes in brushing technique, becoming more vigilant with toothbrushing and avoiding brushing parts of their mouth due to the associated pain. The potential consequence of advancing gingivitis symptoms, fear of losing teeth, and feeling unhealthy or unclean were the most common perceived impacts. Study participants also reported avoiding laughing, covering their mouth, and using other methods to hide their symptoms in social situations. The authors concluded that "all gum health conditions should be considered in OHRQoL-related discussions."[25] A recent workshop in Latin America recommended that health authorities develop "policies and programs for maintaining oral health and avoiding periodontitis through the effective management of gingivitis and promotion of healthy lifestyles at both population and individual levels."[26] Thus, it is important that dentifrices be developed and evaluated with proven clinical efficacy against oral biofilm and gingivitis with the ultimate goal of patient acceptance and use.

A new SnF_2 dentifrice stabilized with nitrate and phosphates was compared to a regular fluoride dentifrice in a phase III, single-center, double-blind, parallel-group randomized clinical trial that evaluated the control of dental plaque and gingivitis over a

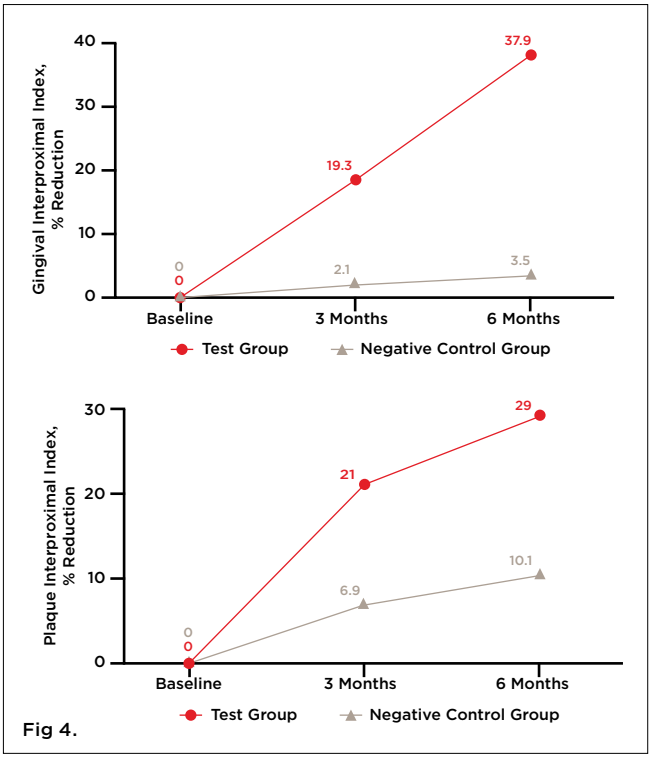

Fig 4. Percentage reduction relative to baseline for gingival interproximal index (top) and plaque interproximal index (bottom).

6-month period. There were no significant differences between the participants in the two treatment groups with respect to age, sex, or baseline gingival health. The results showed that after 6 months of twice-daily brushing, all plaque and gingivitis indices improved relative to their baseline values for both the test dentifrice and the negative control dentifrice with the exception of the gingival interproximal index for the negative control dentifrice.

Previous studies have shown that twice-daily toothbrushing with a regular fluoride dentifrice improves gingival health by the removal of dental plaque and the concurrent reduction in gingivitis.[12,13] Such is the case in this study as well with the negative control dentifrice demonstrating a reduction from baseline in almost all the plaque and gingivitis indices at 6 months. SNaP demonstrated a benefit beyond that of the regular fluoride dentifrice and was clinically proven to be significantly superior to the regular fluoride dentifrice in terms of the removal of dental plaque and the improvement in gingival health after 3 months and 6 months of twice-daily brushing as measured by all the plaque and gingival indices. Included in these results are the findings from hard-to-reach or interproximal areas in the oral cavity.

Finally, there was a substantial reduction of 90% in the gingival severity index (bleeding) for the test dentifrice as compared to the negative control dentifrice after 6 months' use. All participants using the test dentifrice showed improved gum health after 6 months. These findings along with the other studies[10,18-20] in this current publication support the fact that SnF_2 is both stable and bioavailable from this new 0.454% stannous fluoride dentifrice stabilized with nitrate and phosphates (SNaP technology), providing benefits in addressing many common oral care problems beyond those achieved by brushing with a regular fluoride dentifrice.

Conclusion

As compared to baseline and to a regular fluoride dentifrice, twice-daily brushing with a new 0.454% stannous fluoride dentifrice stabilized with nitrate and phosphates provides significant clinical benefit through the control of dental plaque and improvement of gingival health over 6 months. This SNaP dentifrice offers a new therapeutic and preventive option for dental practitioners to recommend to their patients.

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DISCLOSURES

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DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Effect of a Stannous Fluoride Toothpaste Stabilized With Nitrate and Phosphates (SNaP) on Dentin Hypersensitivity: In Vitro Study and Randomized Controlled Trial

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Abstract: *Background:* Dentin hypersensitivity is a global oral health concern. This in vitro study and clinical evaluation tested the efficacy of 0.454% stannous fluoride toothpaste stabilized with nitrate and phosphates (SNaP) to occlude dentin and reduce dentin hypersensitivity. *Methods:* Human dentin specimens were treated with test and control toothpaste slurries and evaluated for dentin occlusion. In a phase III randomized controlled trial, eligible participants were randomized to SNaP toothpaste (test group), a potassium nitrate desensitizing dentifrice (positive control), or a non-desensitizing sodium monofluorophosphate dentifrice (negative control). Baseline, day 1, day 3, and day 7 dentin hypersensitivity was assessed using tactile and air blast stimuli. Mean tactile and air blast hypersensitivity scores were calculated for each treatment group. For statistical analysis, significance was set to $P \leq .05$. *Results:* In vitro mean percent tubule occlusion for test and control samples were 86% and 35%, respectively ($P < .05$). One-hundred-twenty participants completed the clinical trial. After 7 days of product use, relative to the positive control and negative control groups, the test group exhibited significant reduction in tactile hypersensitivity (79.8% and 90.2%, respectively; $P < .001$) and reduction in air blast hypersensitivity (47.1% and 47.9%, respectively; $P < .001$). SNaP significantly reduced hypersensitivity pain after 1, 3, and 7 days. *Conclusions:* In vitro and clinical evaluation results indicate that SNaP is highly effective in coating the dentin surface, occluding exposed dentin tubules, and offering sensitivity relief from the first day of use, a benefit that continues to improve with use. *Practical Implications:* This multi-benefit formula reduces dentin hypersensitivity, thereby improving the daily lives of patients, promoting better oral health, and potentially helping patients avoid more serious dental problems in the future.

Dentin hypersensitivity is a global oral health concern, a significant challenge within dental practice, and it can negatively impact patients' quality of life.[1-3] Dentin's innate sensitivity to stimuli is not an issue when protected by enamel and cementum; however, when dentin hypersensitivity develops due to enamel erosion, gum recession, or other factors, the tubules of dentin become exposed.[2] Prevalence estimates of dentin hypersensitivity vary widely by population and healthcare setting, but a fixed-effects meta-analysis reported a population prevalence estimate of 11.5% (95% CI [11.3, 11.7]).[1] Those impacted by dentin hypersensitivity report tooth pain when dentin is exposed to chemical, thermal, or other stimuli.[2,4] This discomfort can limit a patient's dietary selections and negatively impact one's ability

to follow recommended oral care routines.[5]

Consensus-based recommendations for the management of dentin hypersensitivity have warned that the condition is underdiagnosed, associated with poor health outcomes, and lacks "widespread availability of noninvasive, efficacious, and inexpensive" treatment.[4] Toothpaste has been increasingly trialed with active ingredients formulated to lessen the discomfort and burden of dentin hypersensitivity.[6] There are two treatment strategies to relieve hypersensitivity: agents that occlude dentin tubules, blocking the source of the discomfort, and agents that disrupt the neural pain response.[2] For the latter, potassium salts are used as active agents in antisensitivity toothpaste to disrupt the pain response; however, these products can take several weeks of use for patients to feel relief.[7,8]

Dentin occluding agents offer an alternative approach in which hypersensitivity is addressed at its root cause as opposed to treating the symptoms.[2,6] Stannous fluoride forms insoluble precipitates to occlude dentin tubules. An in vitro study found that dentin specimens treated with a stannous fluoride toothpaste had significantly more occluded dentin tubules than dentin specimens treated with a non-stannous fluoride formula containing sodium monofluorophosphate.[9] When the same products were tested in a clinical trial of patients with dentin hypersensitivity, the test toothpaste provided significant improvements in tactile and air blast hypersensitivity scores compared to the negative control toothpaste at 4- and 8-week intervals.[9]

Dentin occluding agents offer an alternative approach in which hypersensitivity is addressed at its root cause as opposed to treating the symptoms. Stannous fluoride forms insoluble precipitates to occlude dentin tubules.

The following in vitro study and clinical evaluation builds on this work to test the efficacy of a 0.454% stannous fluoride toothpaste stabilized with nitrate and phosphates (SNaP) to reduce dentin hypersensitivity compared to a potassium-based dentifrice and to a non-desensitizing regular 0.76% sodium monofluorophosphate dentifrice after twice-daily brushing for 1, 3, and 7 days. SNaP is formulated to offer antigingivitis, anticaries, extrinsic tooth stain removal, antisensitivity, and antibacterial benefits, as well as reduced oral malodor.[10-13]

Material and Methods

In Vitro Methods

Products Tested: The test group was treated with the SNaP toothpaste (Colgate-Palmolive Co., colgatepalmolive.com). The control group was treated with a negative control toothpaste containing 0.76% sodium monofluorophosphate (Colgate-Palmolive Co.).

In Vitro Assessment of Dentin Occlusion: For the in vitro assessment of dentin occlusion, confocal microscopy coupled with image analysis software (Leica Map version 7.1, Leica Microsystems, leica-microsystems.com) and visual inspection was utilized to quantify the occlusion of treated dentin specimens.

Dentin Sample Preparation: Human teeth were mounted on a saw (IsoMet® High Speed Pro, Buehler, buehler.com) and cross-sectioned into 700-µm thick slices. Cut dentin specimens were then sanded and polished on a polishing grinder (EcoMet® III, Buehler) with a polishing cloth (Buehler). Specimens were sonicated in deionized water, then etched with 1% citric acid, dried, and stored on wet tissue.

Treatment Procedure: The dentin surfaces of three specimens (per tested toothpaste) were brushed for 30 seconds, using a microbrush and toothpaste slurry. Toothpaste slurries were created using one part phosphate buffered saline (PBS) to three parts tested toothpaste. Samples were allowed to sit for 15 minutes at room temperature, placed in 10 milliliters of PBS, stirred at 125 to 130 revolutions per minute for 15 minutes, rinsed, and dried. The procedure was completed five times.

Measurement and Quantification of Occlusion: Five regions were marked on the non-sampling side of each dentin specimen. Each sample was then mounted on a glass slide with tape for imaging (DCM 3D Microscope, Leica Microsystems) before treatment (for baseline). Leica Map version 7.1 was used and coupled with an imaging and analysis program to achieve the calculation from images.[14] Confocal images of each sample region were captured at 10-times magnification to identify the starting point, and acquired and reported pictures at 150-times magnification. Mean percentage of occlusion was calculated on three dentin samples for each toothpaste, with a total of 15 data points analyzed for each tested toothpaste. Percent occlusion was quantified based on the total area occupied by the open tubules of the untreated specimens against the area of any open tubules of the treated specimens. The percentage of occlusion was calculated as follows: $100 - ((R_2/R_1) \times 100)$ where R_1 represents the area of open tubules for untreated dentin and R_2 represents the area of open tubules for treated dentin.[9,14]

Clinical Assessment Trial Design

The clinical assessment was a phase III randomized, single-center, double-blind, three-cell parallel-group clinical study.

Products Tested: The test group used the SNaP toothpaste (Colgate-Palmolive Co.). The first control group used a positive control desensitizing toothpaste containing 0.24% sodium fluoride and 5% potassium nitrate (GlaxoSmithKline Co., gsk.com). The second control group used a negative control toothpaste, a non-desensitizing regular 0.76% sodium monofluorophosphate toothpaste (Colgate-Palmolive Co.).

Ethics: The study (US IRB2020CP/03) was reviewed and approved by the U.S. Investigational Review Board, Inc. (U.S. IRB, Inc®), 6400 SW 72 Court, Miami, Florida 33143. All study participants signed an informed consent form.

Study Setting and Location: Healthy male and female participants were enrolled in the Costa Mesa, California, area. The recruitment period was from September 28, 2020, to September 29, 2020. The study period was from September 28, 2020, to October 21, 2020. At the clinical site, all eligible individuals were assessed by means of tactile and air blast stimuli at all study timepoints (baseline, day 1, day 3, and day 7) by the same examiner.

Participant Inclusion and Exclusion Criteria: Participants were eligible for the study if they met the following inclusion criteria: willing to sign an informed consent form; male or female adults between the ages of 18 and 70 (inclusive); in general good health as determined by the study investigators; able to participate for the full duration of the study (7 days); a minimum of two hypersensitive teeth that were anterior to the molars and demonstrated dentin exposure due to cervical erosion/abrasion or gingival recession; with qualifying dentin hypersensitivity response to tactile stimuli applied to the cervical surface as defined by a response score between 10 and 50 grams of force (Yeaple Probe, XiniX Research Inc., yeapleprobe.com); and a qualifying dentin hypersensitivity response to air blast stimuli applied for 1 second to the cervical surface (gingiva-facial third) as defined by a score of 2 or 3 on the Schiff cold air sensitivity scale.[15]

Participants were excluded from the study if any of the following applied: gross oral pathology, chronic disease, and/or history of allergies to any of the test products; use of any desensitizing oral care products or treatment within the past 3 months; advanced periodontal disease and/or treatment for periodontal disease within the past 12 months; hypersensitive teeth with a mobility greater than one; teeth with extensive/defective restorations or with suspected pulpitis, caries, cracked enamel, or used as abutments for removable partial dentures; current use of anticonvulsants, antihistamines, antidepressants, sedatives, tranquilizers, anti-inflammatory drugs, or daily analgesics; current participation in any oral clinical studies; self-reported pregnancy or breastfeeding; allergies to oral care products or personal care consumer products or their ingredients, or a medical condition(s) that prohibits not eating/drinking for 4 hours.

Sample Size: The sample size of 40 per group (120 total) was determined based on a standard deviation (SD) for the response measure tactile sensitivity (or air blast) of 3.34 (or 0.31), a significance level of $\alpha = 0.05$, a 10% attrition rate, and an 80% level of power. The study was powered to detect a minimal statistically significant difference between the study group means of 20%.

Randomization of Treatments and Treatment Assignment: Study participants were provided with an identification number in chronological order as they were enrolled in the study. These numbers were randomly pre-assigned to a treatment group following a computer-generated randomization list. The participants enrolled in the study were randomly assigned to one of three study groups in such a way that neither the examiner nor the study participant was aware of the individual's treatment group.

Intervention: All participants were provided with their assigned toothpaste and a soft-bristled adult toothbrush and were instructed to brush for 2 minutes twice per day (once in the morning and once in the evening) for 7 days. Participants discontinued use of any other oral hygiene practices during the study period, but no restrictions were placed on dietary or smoking habits.

Clinical Scoring Procedures: Study participants were instructed to refrain from any oral hygiene procedure and/or chewing gum for 8 hours, and from eating and/or drinking for 4 hours prior to each scheduled visit (baseline, day 1, day 3, and day 7) to the clinical site. Participants were screened by the dental examiner.

Tactile Hypersensitivity Assessment: Participants' tactile sensitivity was assessed by use of the Model 200A Electronic Force-Sensing Probe developed by Yeaple Research of Pittsford, New York. The application of this probe for dentin sensitivity testing utilizing a #19 explorer tip at a preset force measured in grams was employed.

Teeth were evaluated for tactile hypersensitivity in the following manner[16,17]: (1) Participants were instructed to respond at the point when they first experienced discomfort. (2) The explorer tip of the probe was applied to the buccal surface of each sensitive tooth at the cementoenamel junction. (3) The explorer tip was stroked perpendicular to the tooth beginning at a preset force of 10 grams and increasing by 10-gram increments until the participant experienced discomfort or 50 grams of force was applied. If there was no indication of discomfort upon application of 50 grams of force, the tooth was deemed nonsensitive to tactile stimulation and ineligible for inclusion in the study.

Air Blast Hypersensitivity Assessment: Air blast evaluations were conducted approximately 5 minutes after tactile evaluation. Teeth that were identified as sensitive and which demonstrated abrasion, erosion, and/or gingival recession were evaluated in the following manner: (1) The sensitive tooth was isolated from the adjacent teeth (mesial and distal) by the placement of the examiner's fingers over the adjacent teeth. (2) Air was delivered/ejected from a standard dental unit air syringe at 60 PSI (±5 PSI) and 70°F (±3°F). (3) The air was directed at the exposed buccal surface of the sensitive tooth for 1 second from a distance of approximately 1 centimeter. The Schiff cold sensitivity scale was used to assess the participant's response to this stimulus.[15] Sensitivity was scored as follows: 0 = tooth/participant does not respond to air stimulus; 1 = tooth/participant responds to air stimulus but does not request discontinuation of stimulus; 2 = tooth/participant responds to air stimulus and requests discontinuation or moves from stimulus; 3 = tooth/participant responds to air stimulus, considers stimulus to be painful, and requests discontinuation of the stimulus.

Monitoring/Reporting of Adverse Events

All clinical complaints, symptoms, or signs that met the adverse event definition were recorded on a case report form. Adverse events were assessed by the investigator or designee for severity, relationship to the study product, possible etiologies, and whether the event met the criteria as a serious adverse event.

Statistical Methods: In Vitro Study

Data analysis was conducted by using statistical software (Minitab

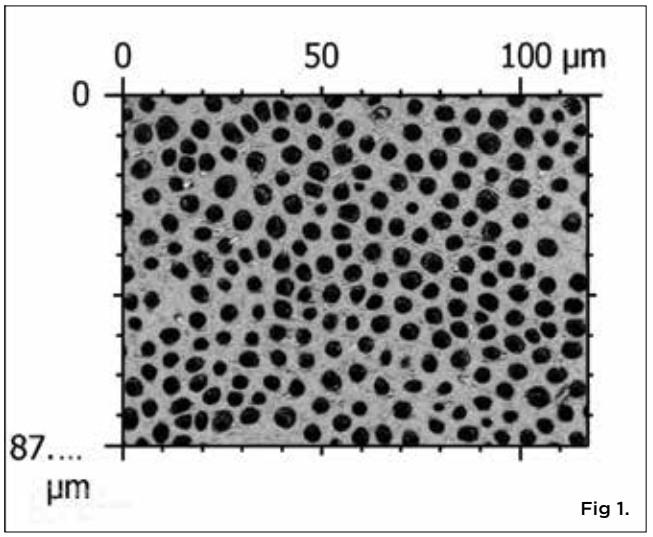

Fig 1.

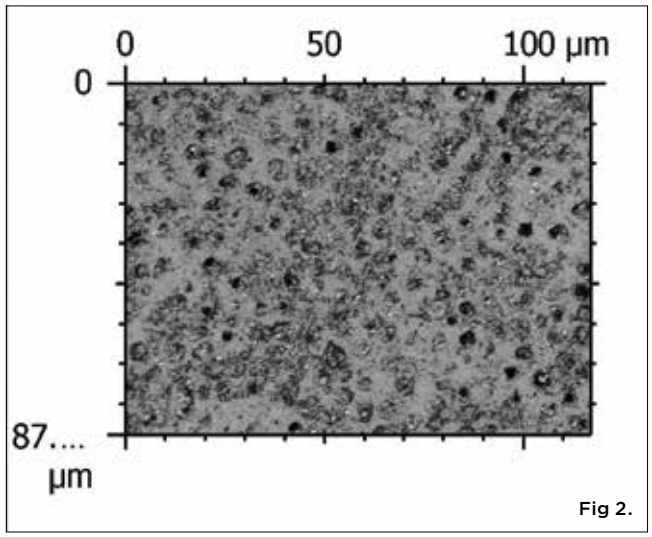

Fig 2.

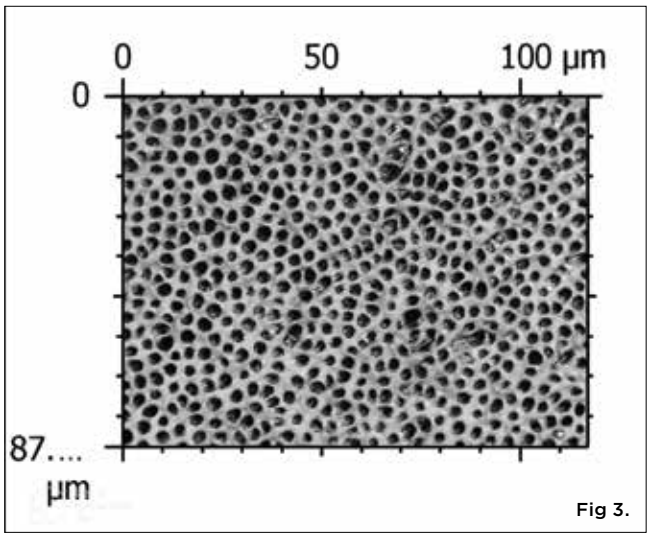

Fig 3.

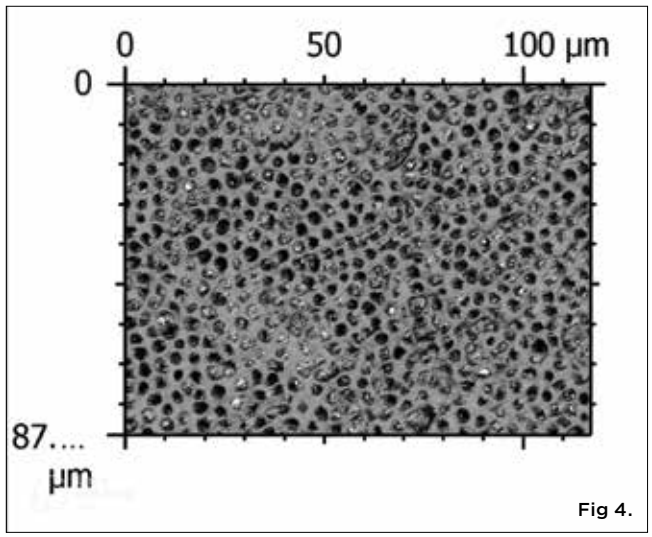

Fig 4.

Fig 1 through Fig 4. Dentin specimens before and after toothpaste treatment. Representative confocal images of dentin specimens at 150x magnification for: (Fig 1) test toothpaste, before treatment; (Fig 2) test toothpaste, after treatment; (Fig 3) control toothpaste, before treatment; (Fig 4) control toothpaste, after treatment.

version 18.1, Minitab, minitab.com) with a student's t-test comparing the mean percentage of occlusion for each of the toothpastes. Differences between treatments were statistically significant if the *P* value was less than or equal to .05.

Statistical Methods: Clinical Assessment

Data analysis was performed on tactile and air blast hypersensitivity assessments by using statistical software (Minitab version 18.1). Comparisons of the study treatment group demographics were analyzed using a chi-square test to assess gender and an independent student's t-test for age. Comparison of the treatment groups with respect to baseline tactile and air bast hypersensitivity, and baseline compared to follow-up, were analyzed with an independent student's t-test. The between-treatment comparisons with respect to baseline-adjusted tactile and air blast hypersensitivity at the follow-up examinations were analyzed using an analysis of covariance (ANCOVA) model. Differences within and between treatments were statistically significant if *P* value was less than or equal to .05.

Results

In Vitro Results

Confocal microscopy images (representative images are shown in Figure 1 through Figure 4) were acquired by means of confocal microscopy of dentin specimens treated with test and negative control toothpastes. Confocal images of the test specimens before and after five treatments (Figure 1 and Figure 2) as well as the control specimens before and after five treatments (Figure 3 and Figure 4) were collected. The visual inspection aligned with the quantified mean percent occlusion for both treatment groups. Dentin specimens treated with the test toothpaste had 86% occlusion, whereas the negative control toothpaste had 35% occlusion. The difference between these two products was significantly different at a 95% confidence level according to results with a two-sample t-test ($P < .05$).

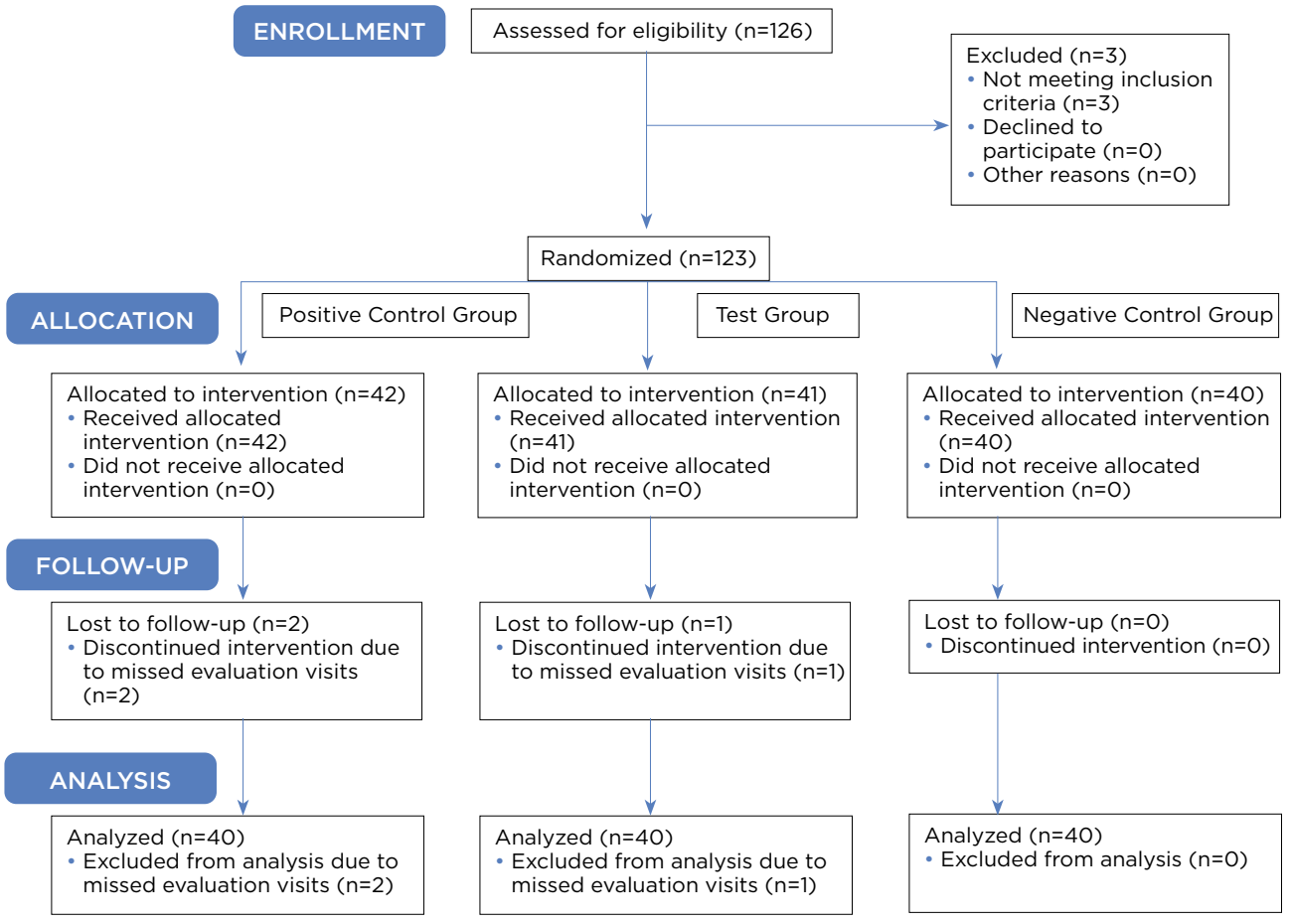

Fig 5. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study population for the 7-day dentin hypersensitivity clinical study.

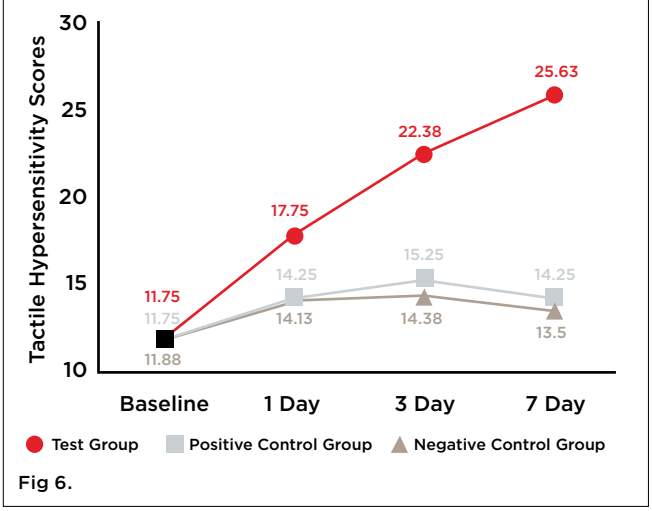

Fig 6. Tactile hypersensitivity scores between groups over time. Unadjusted subject mean Yeaple tactile hypersensitivity scores (grams) at baseline, 1-day, 3-day, and 7-day examinations.

Clinical Trial Results

One-hundred-twenty (120) participants complied with the protocol and completed the clinical study (Figure 5). The trial stopped at the end of the study period. Mean age and ranges are reported in Table 1. No statistically significant differences were indicated among the three groups at baseline with respect to either tactile hypersensitivity ($P = .980$) or air blast hypersensitivity ($P = .982$). No subgroup analyses were performed. Unadjusted tactile and air blast scores are reported in Table 2, Figure 6, and Figure 7.

Tactile Hypersensitivity: The test toothpaste, SNaP, provided statistically significant improvements in dentin hypersensitivity after 1, 3, and 7 days (Figure 6).

After 1 day of product use, the percent improvements in tactile hypersensitivity from baseline were 51.1% ($P < .001$) for the test group, 21.3% ($P = .001$) for the positive control group, and 18.9% ($P < .001$) for the negative control group (Table 3). Relative to participants in the positive control and negative control groups, those in the test group exhibited statistically significant improvements of 24.5% ($P < .001$) and 26.4% ($P < .001$), respectively, in tactile hypersensitivity scores (Table 3).

TABLE 1

Summary of Age and Gender for Subjects Who Completed the Clinical Study

TREATMENT GROUP	NUMBER OF SUBJECTS[1]			AGE[1]	
	Male, N (%)	Female, N (%)	Total	Mean (SD)	Range
Test	14 (35)	26 (65)	40	42.98 (13.90)	18–69
Positive Control	15 (37.5)	25 (62.5)	40	40.33 (12.49)	20–66
Negative Control	16 (40)	24 (60)	40	42.02 (13.91)	18–65
All Treatment Groups	45	75	120	41.77 (13.38)	18–69

1 No statistically significant differences were indicated among the three treatment groups with respect to either gender (P = .899) or age (P = .672) characteristics.
SD = standard deviation

TABLE 2

Subject Mean (SD) Tactile and Air Blast Hypersensitivity Scores at Baseline, 1-Day, 3-Day, and 7-Day Examinations for Subjects Who Completed the Clinical Study

PARAMETER	TREATMENT GROUP	n	BASELINE MEAN (SD)[1]	DAY 1 MEAN (SD)	DAY 3 MEAN (SD)	DAY 7 MEAN (SD)
Tactile Hypersensitivity	Test	40	11.75 (3.11)	17.75 (4.66)	22.38 (5.19)	25.63 (5.68)
	Positive Control	40	11.75 (3.11)	14.25 (4.88)	15.25 (5.18)	14.25 (5.83)
	Negative Control	40	11.88 (3.34)	14.13 (4.07)	14.38 (4.27)	13.50 (5.68)
Air Blast Hypersensitivity	Test	40	2.78 (0.34)	2.09 (0.41)	1.64 (0.47)	1.35 (0.40)
	Positive Control	40	2.79 (0.34)	2.63 (0.42)	2.43 (0.53)	2.55 (0.52)
	Negative Control	40	2.78 (0.34)	2.58 (0.46)	2.49 (0.42)	2.59 (0.54)

1 No statistically significant differences were indicated among the three treatment groups at baseline with respect to either tactile hypersensitivity (P = .980) or air blast hypersensitivity (P = .982) scores.
SD = standard deviation

After 3 days of product use, the percent improvements in tactile hypersensitivity from baseline were 90.5% (P < .001) for the test group, 29.8% (P < .001) for the positive control group, and 21.0% (P < .001) for the negative control group (Table 4). The test group also exhibited statistically significant improvements when compared to the positive control and negative control groups: 46.6% (P < .001) and 56.1% (P < .001), respectively (Table 4).

After 7 days of product use, the percent improvements in tactile hypersensitivity from baseline were 118.1% (P < .001) for the test group, 21.3% (P < .001) for the positive control group, and 13.6% (P = .057) for the negative control group (Table 5). Relative to participants in the positive control and negative control groups, those in the test group exhibited statistically significant improvement in tactile hypersensitivity (79.8% and 90.2%, respectively; P < .001) (Table 5).

Air Blast Hypersensitivity: The test toothpaste, SNaP, provided

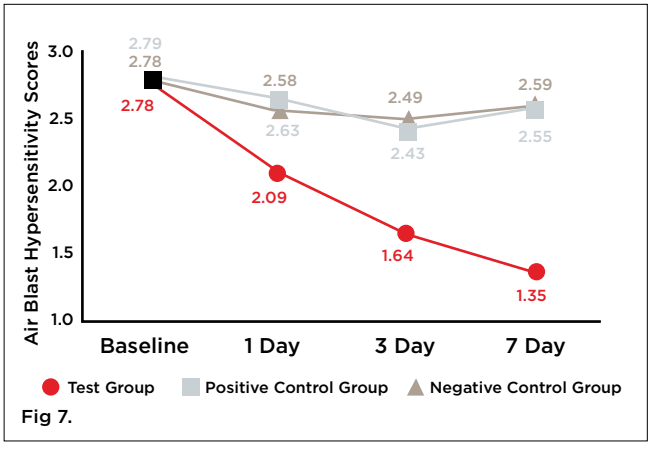

Fig 7.

Fig 7. Air blast hypersensitivity scores between groups over time. Unadjusted subject mean Schiff cold hypersensitivity scores at baseline, 1-day, 3-day, and 7-day examinations.

TABLE 3

Baseline-Adjusted Subject Mean (SE) Tactile and Air Blast Hypersensitivity Scores at 1-Day Examination

PARAMETER	TREATMENT GROUP	n	ADJUSTED 1-DAY MEAN (SE)	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISON			
						vs. Positive Control Group		vs. Negative Control Group	
				Percent Change[1]	Sig.[2]	Percent Difference[3]	Sig.[5]	Percent Difference[4]	Sig.[5]
Tactile Hypersensitivity	Test	40	17.78 (0.63)	51.1%	*P* < .001	24.5%	*P* < .001	26.4%	*P* < .001
	Positive Control	40	14.28 (0.63)	21.3%	*P* = .001	-----	-----	1.5%	*P* = .968
	Negative Control	40	14.07 (0.63)	18.9%	*P* < .001	-----	-----	-----	-----
Air Blast Hypersensitivity	Test	40	2.09 (0.06)	24.8%	*P* < .001	20.2%	*P* < .001	19.0%	*P* < .001
	Positive Control	40	2.62 (0.06)	5.7%	*P* = .003	-----	-----	-1.6%	*P* = .872
	Negative Control	40	2.58 (0.06)	7.2%	*P* = .002	-----	-----	-----	-----

1 Percent change exhibited by the 1-day mean relative to the baseline mean. A positive value indicates an improvement in hypersensitivity scores at the 1-day examination.
2 Significance of paired t-test comparing the baseline and 1-day examinations.
3 Difference between the adjusted 1-day means expressed as a percentage of the adjusted 1-day mean for the Positive Control group. A positive value indicates an improvement in hypersensitivity scores for row heading relative to the Positive Control group.
4 Difference between the adjusted 1-day means expressed as a percentage of the adjusted 1-day mean for the Negative Control group. A positive value indicates an improvement in hypersensitivity scores for the row heading relative to the Negative Control group.
5 Significance of post-ANCOVA Tukey's multiple comparison test of baseline-adjusted 1-day means.
SE = standard error, Sig. = significance

statistically significant improvements in air blast hypersensitivity after 1, 3, and 7 days (Figure 7).

After 1 day of product use, the percent reductions in air blast hypersensitivity from baseline were 24.8% ($P < .001$) for the test group, 5.7% ($P = .003$) for the positive control group, and 7.2% ($P = .002$) for the negative control group (Table 3). Relative to participants in the positive control and negative control groups, those in the test group exhibited statistically significant improvements of 20.2% ($P < .001$) and 19.0% ($P < .001$), respectively, in air blast sensitivity scores after 1 day of product use (Table 3).

After 3 days of product use, the percent reductions in air blast hypersensitivity from baseline were 41.0% ($P < .001$) for the test group, 12.9% ($P < .001$) for the positive control group, and 10.4% ($P < .001$) for the negative control group (Table 4). Relative to participants in the positive control and negative control groups, those in the test group exhibited statistically significant reductions of 32.2% ($P < .001$) and 34.1% ($P < .001$), respectively, in air blast hypersensitivity scores after 3 days of product use (Table 4).

After 7 days of product use, the percent reductions in air blast hypersensitivity from baseline were 51.4% ($P < .001$) for the test group, 8.6% ($P = .003$) for the positive control group, and 6.8% ($P = .038$) for the negative control group (Table 5). Finally, after 7 days of product use, relative to participants in the positive control and negative control groups, those in the test group exhibited statistically significant reductions of 47.1% ($P < .001$) and 47.9% ($P < .001$), respectively, in air blast hypersensitivity scores (Table 5).

The results highlight the significant sensitivity reductions provided by stannous fluoride toothpaste occlusion technology compared to a potassium nitrate desensitizing toothpaste.

Adverse Events: No adverse events were observed by the investigator or reported by the study participants.

Discussion

Confocal microscopy confirmed that the SNaP toothpaste was highly effective in coating the dentin surface and occluding dentin tubules. Image analysis of the confocal images helped verify that 86% of the tubules were occluded after in vitro treatment with SNaP. The negative control toothpaste, resulting in 35% occlusion after treatment, was less effective. Visual inspection of confocal images helped confirm that most of the tubules were occluded fully after treatment with the stannous fluoride toothpaste, whereas most of the dentin tubules treated with the negative control toothpaste remained open. In vitro results alone are promising but do not prove efficacy in a real-world setting.

However, in addition to the dentin occlusion confirmed in

TABLE 4

Baseline-Adjusted Subject Mean (SE) Tactile and Air Blast Hypersensitivity Scores at 3-Day Examination

PARAMETER	TREATMENT GROUP	n	ADJUSTED 1-DAY MEAN (SE)	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISON			
						vs. Positive Control Group		vs. Negative Control Group	
				Percent Change[1]	Sig.[2]	Percent Difference[3]	Sig.[5]	Percent Difference[4]	Sig.[5]
Tactile Hypersensitivity	Test	40	22.39 (0.74)	90.5%	*P* < .001	46.6%	*P* < .001	56.1%	*P* < .001
	Positive Control	40	15.27 (0.74)	29.8%	*P* < .001	-----	-----	6.5%	*P* = .650
	Negative Control	40	14.34 (0.74)	21.0%	*P* < .001	-----	-----	-----	-----
Air Blast Hypersensitivity	Test	40	1.64 (0.07)	41.0%	*P* < .001	32.2%	*P* < .001	34.1%	*P* < .001
	Positive Control	40	2.42 (0.07)	12.9%	*P* < .001	-----	-----	2.8%	*P* = .770
	Negative Control	40	2.49 (0.07)	10.4%	*P* < .001	-----	-----	-----	-----

1 Percent change exhibited by the 3-day mean relative to the baseline mean. A positive value indicates an improvement in hypersensitivity scores at the 3-day examination.
2 Significance of paired t-test comparing the baseline and 3-day examinations.
3 Difference between the adjusted 3-day means expressed as a percentage of the adjusted 3-day mean for the Positive Control group. A positive value indicates an improvement in hypersensitivity scores for row heading relative to the Positive Control group.
4 Difference between the adjusted 3-day means expressed as a percentage of the adjusted 3-day mean for the Negative Control group. A positive value indicates an improvement in hypersensitivity scores for the row heading relative to the Negative Control group.
5 Significance of post-ANCOVA Tukey's multiple comparison test of baseline-adjusted 3-day means.
SE = standard error, Sig. = significance

the in vitro results, SNaP provided hypersensitivity relief in a clinical evaluation. The randomized trial was well controlled and utilized double-blinding methodology. SNaP provided a statistically significant improvement in hypersensitivity after 1, 3, and 7 days of product use as compared to a commercially available potassium-based toothpaste and a non-desensitizing regular fluoride toothpaste containing 0.76% sodium monofluorophosphate. The clinical results highlight the significant and faster sensitivity reductions (1 day) provided by stannous fluoride toothpaste occlusion technology compared to a potassium nitrate desensitizing toothpaste, which did not show significant reductions from the negative control even after 7 days. For both tactile and air blast hypersensitivity scores, the percentage difference for the SNaP toothpaste test group increased as a function of time. While the sample was sufficiently sized to show clinically meaningful results, it was a single-site study and results may not be applicable to all patient groups.

Dentin hypersensitivity is known to interfere with recommended oral care routines. Cyclically, poor oral care routine then contributes to periodontal diseases associated with root exposure and root caries, which then increases the risk of incidence or worsening of dentin hypersensitivity.[5,18,19] A desensitizing, tubule-occluding toothpaste that reduces hypersensitivity in less than a week interrupts this cycle and, in alignment with consensus-based recommendations,[4] provides low-cost and widely available treatment for dentin hypersensitivity patients.

Conclusions

In vitro results indicate that SNaP toothpaste was highly effective in coating the dentin surface and occluding exposed dentin tubules, which is the root cause of dentin hypersensitivity. SNaP provided a statistically significant reduction in dentin hypersensitivity after 1, 3, and 7 days of product use as compared to a commercially available desensitizing potassium-based toothpaste and a regular fluoride toothpaste containing 0.76% sodium monofluorophosphate. This multi-benefit SNaP toothpaste holds promise to improve oral care routines and, ultimately, the oral health and quality of life for patients suffering from dentin hypersensitivity.

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DISCLOSURES

This clinical trial was supported by funding from the Colgate-

TABLE 5

Baseline-Adjusted Subject Mean (SE) Tactile and Air Blast Hypersensitivity Scores at 7-Day Examination

PARAMETER	TREATMENT GROUP	n	ADJUSTED 1-DAY MEAN (SE)	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISON			
						vs. Positive Control Group		vs. Negative Control Group	
				Percent Change[1]	Sig.[2]	Percent Difference[3]	Sig.[5]	Percent Difference[4]	Sig.[5]
Tactile Hypersensitivity	Test	40	25.64 (0.90)	118.1%	*P* < .001	79.8%	*P* < .001	90.2%	*P* < .001
	Positive Control	40	14.26 (0.90)	21.3%	*P* < .001	-----	-----	5.8%	*P* = .812
	Negative Control	40	13.48 (0.90)	13.6%	*P* = .057	-----	-----	-----	-----
Air Blast Hypersensitivity	Test	40	1.35 (0.08)	51.4%	*P* < .001	47.1%	*P* < .001	47.9%	*P* < .001
	Positive Control	40	2.55 (0.08)	8.6%	*P* = .003	-----	-----	1.5%	*P* = .917
	Negative Control	40	2.59 (0.08)	6.8%	*P* = .038	-----	-----	-----	-----

1 Percent change exhibited by the 7-day mean relative to the baseline mean. A positive value indicates an improvement in hypersensitivity scores at the 7-day examination.
2 Significance of paired t-test comparing the baseline and 7-day examinations.
3 Difference between the adjusted 7-day means expressed as a percentage of the adjusted 7-day mean for the Positive Control group. A positive value indicates an improvement in hypersensitivity scores for row heading relative to the Positive Control group.
4 Difference between the adjusted 7-day means expressed as a percentage of the adjusted 7-day mean for the Negative Control group. A positive value indicates an improvement in hypersensitivity scores for the row heading relative to the Negative Control group.
5 Significance of post-ANCOVA Tukey's multiple comparison test of baseline-adjusted 7-day means.
SE = standard error, Sig. = significance

Palmolive Company. ClinicalTrials.gov: NCT06244290. The study was reviewed and approved by the U.S. Investigational Review Board, Inc. (U.S. IRB, Inc®), 6400 SW 72 Court, Miami, Florida 33143. The authors YL, SL, CM, GX, EG, YZ, and BG are employees of Colgate-Palmolive Co. CM and GX have patents #US10918580B2 and #US11723846B2 issued to Colgate-Palmolive Co.

DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Stannous Fluoride Toothpaste Stabilized With Nitrate and Phosphates (SNaP) Reduces Oral Malodor: A Randomized Clinical Study

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Abstract: *Background*: Oral malodor, whether from systemic disease, dietary sources, or bacteria in the oral cavity, can negatively impact patients' quality of life. Oral malodor due to bacteria in the oral cavity can be managed by mechanically or chemically removing bacteria. Dentifrices are ideal vehicles to deliver therapeutic active ingredients that promote and maintain oral health since most consumers brush their teeth daily. Consumer preference drives consistency in oral hygiene routine. This study first identified a favorite flavor via consumer flavor testing and then measured the clinical efficacy of the dentifrice with a new flavor formulation to reduce malodor. *Methods*: Consumer testing was conducted via an online product evaluation questionnaire to gauge consumer flavor preferences for stannous fluoride toothpaste stabilized with nitrate and phosphates (SNaP). In a 3-week randomized, single-center, double-blind clinical study, the malodor reduction ability of SNaP was compared to the negative control toothpaste containing 0.76% sodium monofluorophosphate via the organoleptic method. *Results*: Consumer testing was used to determine a winning flavor for the new flavor formulation of SNaP tested in the clinical study. In this study, after 3 weeks of product use, on average, malodor clinical trial subjects (n = 97) randomized into the SNaP group had a 32.7% malodor score reduction from baseline ($P < .001$) 12 hours post-brushing compared to a 9.4% reduction in the negative control group. Relative to the negative control group, the SNaP group had a statistically significant reduction of 25.7% ($P < .001$) in oral malodor via organoleptic scores. *Conclusions*: SNaP toothpaste delivered superior malodor reduction 12 hours post-brushing when compared to a negative control toothpaste. *Practical Implications*: Incorporating therapeutic active ingredients like stannous fluoride into toothpaste is an effective way to deliver oral health benefits, such as caries prevention, reduction in gingivitis and dentin hypersensitivity, and protection against enamel erosion and bad breath.

Oral malodor, also known as halitosis, is a common and manageable condition.[1,2] Prevalence measures vary by population; however, a recent systematic review of oral malodor literature estimated that one in four people have some measure of malodor.[2] Oral malodor is detrimental to patients, as those experiencing it can report a reduction in social function and emotional well-being.[3] Oral malodor is significantly associated with social anxiety.[4] Further, the same bacteria responsible for oral malodor can also cause periodontal disease.[5]

Oral malodor has multiple sources.[2] Systemic issues, such as liver failure, can impact oral malodor, as can dietary choices, smoking, and alcohol consumption.[2] Oral malodor can also be caused by bacteria present in plaque, tongue coating, trapped food, and other substances in the mouth.[5,6] Oral hygiene routines that remove bacteria chemically and mechanically can improve malodor.[7] Dentifrices address oral malodor through multiple routes. First, flavor-formulated dentifrices deliver a pleasant smell to temporarily freshen breath. Also, antimicrobial formulations of dentifrice address the bacterial-related causes of oral malodor.

Dentifrices are ideal vehicles to deliver therapeutic active ingredients that promote and maintain oral health because most consumers brush their teeth daily. Dentifrices have been formulated to have antimicrobial effects that can disrupt the buildup of bacteria leading to malodor.[1] Using stannous fluoride toothpaste when brushing delivers various oral care benefits, including prevention of caries, enamel erosion, and bad breath, reduction of gingivitis and tartar buildup, and decreased dentin hypersensitivity. Stannous fluoride has been found effective in its ability to fight bacteria, including those that produce malodorous compounds.[8-10]

Along with efficacy, taste is another important attribute of oral care products because it is directly linked to consumer preference and adherence to recommended oral healthcare practices. Formulations with pleasing and delightful taste drive consumer exposure to new oral care products and help ensure consumer adherence to oral hygiene regimens compliance.[11,12] To deliver whole-mouth health, a toothpaste formulation needs to contain multiple ingredients that work in synergy and complement each other. These ingredients can impact hedonics and mouth sensation and consequently consumer usage behavior, which can translate into enhanced therapeutic value and efficacy for the consumers.

A novel dentifrice containing 0.454% stannous fluoride stabilized with nitrate and phosphates (SNaP) has been developed to provide a wide spectrum of benefits for the oral cavity.[13] Its efficacy has been clinically proven to deliver whole-mouth health.[10,14-16] However, stannous metal ions can present taste challenges as they impart a lasting unpleasant mouth sensation. Stannous fluoride–containing formulations can have an astringent taste that is mostly noticeable after use of the product. Therefore, consumer acceptability of the SNaP toothpaste flavor must be confirmed in addition to clinical efficacy. The aim of this research was to first select a winning flavor for SNaP development and then measure the oral malodor reduction performance of the SNaP toothpaste.

Materials and Methods

Consumer Testing

A new toothpaste formulation, SNaP, was evaluated via consumer testing in the United States and the United Kingdom. Two new flavor profiles of the SNaP toothpaste formula were compared to the formulation of Colgate Total® (Colgate-Palmolive Co., colgatepalmolive.com) available on the market in the United States and the United Kingdom at the time of consumer testing. Eligible consumer testing participants were men and women aged 18 to 70 (inclusive) responsible for buying and/or choosing the oral care products they use, who were also current users of Colgate Total and brushed their teeth at least once daily.

Screening, placement, and product evaluation was conducted online in a representative national sample of Colgate Total product users. Each subject was given one tube of the assigned toothpaste to try in place of their usual product for 14 days. Consumers completed a questionnaire after the 14 days of in-home product use. The questionnaire asked participants to select their level of agreement with different statements regarding products on a five-point scale where "5" meant the statement "describes the toothpaste you tried completely." The flavor rated most favorably by consumer testing participants was used in the formulation of SNaP toothpaste undergoing clinical testing for efficacy to reduce malodor.

Clinical Investigation

In a randomized, single-center, two-cell, double-blind, parallel clinical study, the SNaP toothpaste was tested for its effectiveness in reducing oral malodor.

Products Tested: The two comparator products were the test toothpaste SNaP, which contains 0.454% stannous fluoride stabilized with nitrate and phosphates, and the negative control toothpaste containing 0.76% sodium monofluorophosphate (Colgate-Palmolive Co.). The consumer-preferred flavor was the flavor utilized in the SNaP toothpaste formulation in the malodor clinical study.

Ethics: The study was reviewed and approved by the Institutional Review Board of China Oral Health Foundation located in the International Building at 18-A South Avenue in Zhongguancun, Haidian District, Beijing, 100081. All participating subjects signed an informed consent form.

Study Setting and Location: The study was conducted at the West China Dental Institute of Chengdu in Chengdu, China. The recruitment period was from October 15 to October 18, 2022. The study period was from June 1 to July 2, 2023.

Participant Inclusion and Exclusion: For study inclusion, subjects had to be aged 18 to 70 (inclusive), in good general health, in good oral health based on self-assessment, available for the full duration of the study, with a baseline mean malodor score between 6.0 and 8.0 (inclusive), a minimum of 20 naturally uncrowned teeth (excluding third molars), and no history of allergies to personal care products or other consumer products or their ingredients.

Subjects were excluded from the study if any of the following applied: participation in any other oral clinical studies during the duration of this study; have full or partial dentures; are pregnant or lactating (breastfeeding); use of tobacco products; history of allergies to common oral care ingredients or oral care products; use of phenolic flavored products such as mint flavored candies and chewing gum the morning of the study and during the sampling periods; immunocompromised individuals (eg, HIV, AIDS, immunosuppressive drug therapy); or unable to abstain from eating or drinking due to medical conditions for the post-use treatment evaluation timepoints.

Sample Size: The sample size of 100 subjects (50 per group)

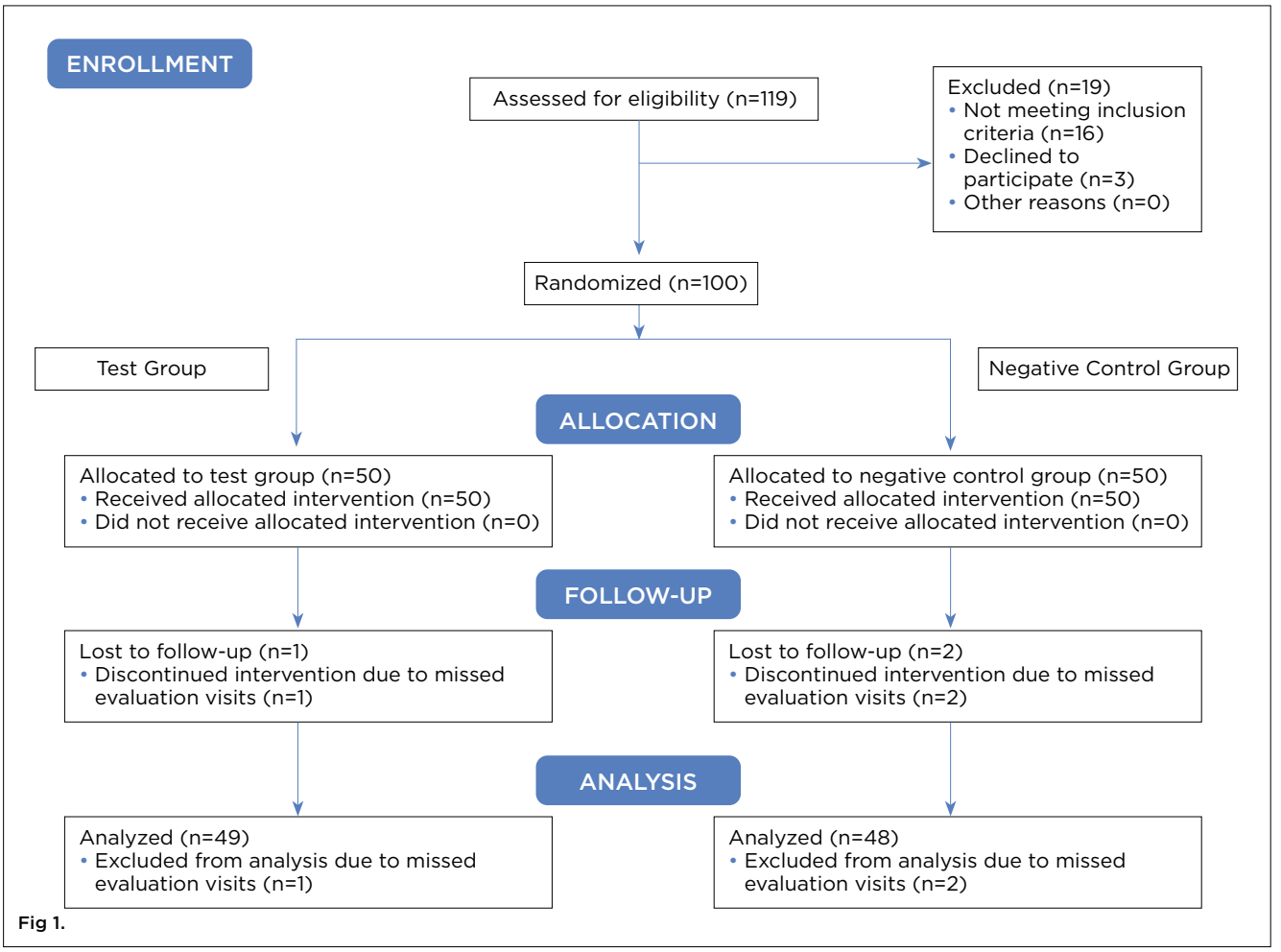

Fig 1.

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study population for clinical evaluation from enrollment to analysis.

was determined based on the overnight organoleptic standard deviation (SD) between products of 2.5, a significance level of $\alpha = 0.05$, a 10% attrition rate, and an 80% level of power. This study was powered to detect a minimal statistically significant difference between study group means of 4.00.

Randomization and Blinding of Treatment: Subjects were randomized into the SNaP test group or negative control group based on a computer-generated list of random numbers. Each group was assigned to a product in a parallel design. The examiners, subjects, and the study statistician were all blinded to product allocation. Products were overwrapped and coded by the sponsor to preserve blinding.

Intervention: All qualified subjects used a regular fluoride toothpaste containing 0.76% sodium monofluorophosphate for daily hygiene (twice daily brushing, once in the morning and once in the evening, for 2 minutes each time) for 7 days. At the end of the 7-day washout period, all subjects returned to the clinical site and were evaluated for their baseline oral malodor by four trained examiners using a hedonic scale from 1 to 9 (described below). Subjects were instructed to refrain from all oral hygiene (brushing, rinsing, and flossing) and eating and drinking for at least 6 hours prior to each scheduled visit for evaluations. Oral malodor evaluations were conducted at baseline and at the 3-week visit. Baseline evaluations were conducted 12 hours post-brushing with the regular fluoride toothpaste. At baseline, the mean of the scores provided by the four judges constituted a subject's baseline oral malodor score. Subjects then brushed with the assigned toothpastes (twice daily, once in the morning and once in the evening, for 2 minutes on each occasion) for the next 3 weeks. At the 3-week evaluation scheduled visit, all subjects were evaluated again for oral malodor 12 hours post-brushing with the assigned toothpastes.

Scoring Procedure: A specially designed screen was used to hide the identities of the judges and subjects and only permit the judges to be exposed to the breath of each individual. When standing in front of this barrier, with the judges on the opposite side, the subjects were instructed to close their mouth, breathe through their nose, and not swallow for 2 minutes. Subjects placed their mouth over the one end of an autoclaved breathing cylinder and breathed gently. Each of the four trained and calibrated examiners placed their nose at the opposite side and scored oral malodor

TABLE 1

Summary of Age and Gender of Subjects Who Completed the Clinical Study

TREATMENT GROUP	NUMBER OF SUBJECTS[1]			AGE, YEARS[1]	
	Male	Female	Total	Mean (SD)	Range
SNaP Group	21	28	49	51.69 (10.82)	28–70
Negative Control Group	21	27	48	51.40 (11.21)	25–68
All Treatment Groups	42	55	97	51.55 (10.96)	25–70

1 No statistically significant difference was indicated between the two treatment groups with respect to gender (P = .929) and age (P = .894) characteristics.
SD = standard deviation

using the following nine-point hedonic scale: 1 = most pleasant; 2 = very pleasant; 3 = moderately pleasant; 4 = slightly pleasant; 5 = neither pleasant nor unpleasant; 6 = slightly unpleasant; 7 = moderately unpleasant; 8 = very unpleasant; 9 = most unpleasant.

Statistical Methods: For each subject at each evaluation timepoint, the hedonic breath-odor scores assigned by the four judges were averaged to yield a single subject-wise score. Statistical analyses were performed on these average organoleptic hedonic scores. Comparisons of the treatment groups with respect to gender were performed using a chi-square analysis and for age an independent t-test.

Comparisons of the treatment groups with respect to baseline organoleptic scores were performed using an independent t-test. Within-treatment comparisons of the baseline versus follow-up organoleptic scores were performed using paired t-tests. Comparisons of the treatment groups with respect to baseline-adjusted organoleptic scores at the follow-up examinations were performed using analysis of covariance (ANCOVA). All statistical tests of hypotheses were two-sided and employed a level of significance of $\alpha = 0.05$.

Results

Consumer Testing

In consumer studies in the United States (n = 164) and the United Kingdom (n = 150), participants preferred the flavor and freshening attributes of the new SNaP toothpaste over the in-market formulation of Colgate Total. They also indicated that this new toothpaste delivered better health-related attributes of "providing long-lasting protection" and "allowing me to be proactive about my oral health." The new formulation was parity on foaming and consistency attributes. Sixty percent of US consumers and 45% of UK consumers indicated a "5" (top score of agreement) regarding the new SNAP toothpaste's freshening attributes; freshness perception and long-lasting fresh breath were rated statistically significantly higher for the new formulation (90% confidence level [CL], two-tailed) compared to the in-market formulation of Colgate Total in both the United States and United Kingdom.

Malodor Reduction

Ninety-seven subjects complied with the protocol and completed the clinical investigation (Figure 1). Three subjects failed to attend all the evaluations and were excluded from the study. The reasons were not product-related. A summary of the gender and age of the study population is presented in Table 1.

For the organoleptic oral malodor assessment, the mean baseline scores were 7.22 for subjects assigned to the SNaP toothpaste group and the negative control group. No statistically significant (P = .994) difference was indicated between the treatment groups with respect to organoleptic scores at baseline.

The baseline-adjusted mean (95% confidence interval [CI]) organoleptic scores evaluated 12 hours post-brushing were 4.86 (95% CI [4.74, 4.98]) for subjects assigned to the SNaP toothpaste group and 6.54 (6.42, 6.66) for subjects assigned to the negative control group (Table 2, Figure 2). The percent reductions from baseline were 32.7% for the SNaP toothpaste group and 9.4% for the negative control group. The percent reductions from baseline observed for the SNaP toothpaste group and negative control group were statistically significant (P < .001).

Use of SNaP toothpaste over a 3-week period provided a statistically significantly greater level of efficacy in controlling oral malodor as compared to the negative control toothpaste. Subjects who brushed with the SNaP toothpaste exhibited a 25.7% reduction in malodor versus the negative control group (P < .001), evaluated 12 hours post-brushing, after 3 weeks of product use via organoleptic scores (Table 2).

Additionally, 85.7% (42 out of 49) of the subjects who brushed with the SNaP toothpaste went into the pleasant breath zone (organoleptic score ≤5) after 3 weeks of product use, while 0.0% (0 out of 48) of the subjects who brushed with the negative control toothpaste containing 0.76% sodium monofluorophosphate did.

Adverse Events

No adverse events were observed by the investigator or reported by subjects.

Discussion

In this randomized clinical study, SNaP toothpaste showed improved malodor reduction benefits compared to the negative control group. Results indicate that the SNaP toothpaste formulation has the ability to reduce malodor by addressing the bacteria biofilm source of malodor. The results demonstrated

TABLE 2

Baseline-Adjusted Mean (SE) Organoleptic Scores at 12 Hours Post-Brushing After 3 Weeks of Product Use for Subjects Who Completed the Clinical Study

TREATMENT GROUP	n	3-WEEK ADJUSTED MEAN (SE)	3-WEEK ADJUSTED 95% CI	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISON		REACHING PLEASANT BREATH ZONE[5]
				Percent Change[1]	Significance[2]	Percent Difference[3]	Significance[4]	Percent
SNaP Group	49	4.86 (0.06)	4.74, 4.98	32.7%	$P < .001$	25.7%	$P < .001$	85.7%
Negative Control Group	48	6.54 (0.06)	6.42, 6.66	9.4%	$P < .001$			0.0%

1 Percent change exhibited by the 3-week mean relative to the baseline mean. A positive value indicates a reduction in organoleptic scores at the 3-week examination.
2 Significance of paired t-tests comparing the baseline and 3-week examinations.
3 Difference between the baseline-adjusted 3-week mean expressed as a percentage of the baseline-adjusted 3-week mean for the Negative Control group. A positive value indicates a reduction in organoleptic scores for the SNaP group relative to the Negative Control group.
4 Significance of ANCOVA comparison of baseline-adjusted 12-hour post-brushing after 3 weeks of product use means.
5 An organoleptic score of ≤5 is considered the pleasant breath zone.
CI = confidence interval, SE = standard error

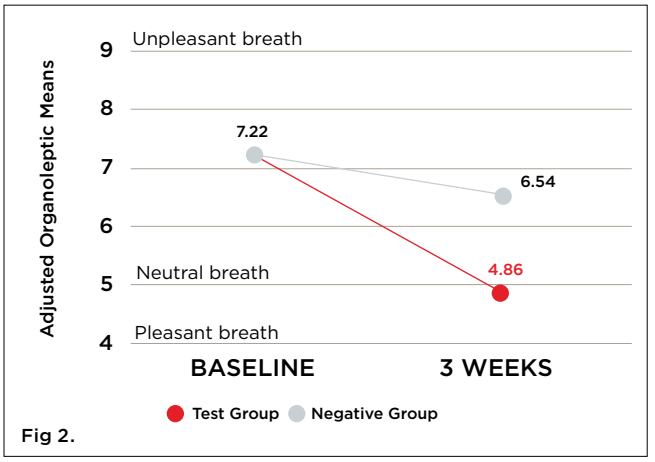

Fig 2. Organoleptic scores comparison between test groups over time. Graph shows subject mean organoleptic scores at baseline and 12 hours post-brushing after 3 weeks of product use.

that the use of SNaP toothpaste over a 3-week period provided a statistically significant greater level of efficacy in controlling malodor for up to 12 hours post-brushing as compared to a commercially available control product. Notably, by the end of the study, more than 85% of participants using SNaP toothpaste had pleasant breath even after 12 hours overnight. Results are consistent with the findings of a recent study testing the efficacy of dentifrices containing stannous fluoride to reduce volatile sulfur compounds, a type of metabolite particularly associated with halitosis.[17] In alignment with the pleasant breath results of the clinical trial, consumer testing also indicated an appealing perception of long-lasting freshness. This could be due to the stannous stabilization system of the SNaP toothpaste.

Stannous fluoride has been found effective in its ability to fight bacteria, including those that produce malodorous compounds.[8-10] Trial results indicate SNaP toothpaste's efficacy to reduce malodor, but clinical benefit cannot be delivered to consumers without consistent use. Flavor is a driver of consumer behavior for toothpaste use. The appealing flavor for the SNaP dentifrice base was preferred by consumers in the United States and the United Kingdom.

The clinical evaluation was a well-controlled, randomized, double-blind trial. It took place, however, at a single site with a specific patient population that suffers from halitosis. Results may not translate to all patient groups but may be highly relevant to other patient groups with halitosis. Future research in certain key areas could enhance the dental profession's understanding and treatment of oral malodor. Comprehensive studies evaluating the long-term efficacy of different treatments could shed light on the sustainability of their results over time. Identifying specific bacteria and bodily processes, as well as the biological mechanisms behind oral malodor, could aid in the development of more targeted and effective treatments. Lastly, performing comparative studies to assess the efficacy of different treatments across various types of oral malodor (physiological and pathological) could help determine the most effective solutions for each specific condition.

Conclusions

The SNaP formulation with the winning flavor was shown to deliver significant malodor reduction benefits as compared to a negative control toothpaste. Based on the results of the consumer and clinical studies, the SNaP formulation delivers

positive esthetic and therapeutic benefits, which are essential to support consumers' everyday use and deliver the benefits of whole-mouth health.

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DISCLOSURES

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DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Efficacy of a Novel Stannous Fluoride Toothpaste Stabilized With Nitrate and Phosphates (SNaP) in Extrinsic Tooth Stain Removal: A Randomized Controlled Trial

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Abstract: *Background*: This study compared the extrinsic tooth stain removal efficacy of a 0.454% stannous fluoride dentifrice stabilized with nitrate and phosphates (SNaP) versus a non-whitening regular fluoride dentifrice (negative control) after 3 and 6 weeks of product use. *Methods*: This phase III, double-blind, randomized, two-cell, parallel-group study was conducted on 80 healthy adults in Puerto Rico. After a baseline tooth stain assessment and oral examinations, study participants were randomly assigned to either the SNaP (test) group or a negative control group. Participants brushed their teeth twice daily for 2 minutes for the 6-week duration. The efficacy for extrinsic tooth stain removal was assessed via Lobene composite stain index, stain area index, and stain intensity index scores for each treatment group at baseline, 3 weeks, and 6 weeks. *Results*: Seventy-eight participants completed the 6-week study. The SNaP dentifrice provided more stain removal relative to baseline tooth stain scores (3-week: 24.4%; 6-week: 35.6%; $P < .001$) and more stain removal relative to the regular fluoride dentifrice (3-week: 24.3%; 6-week: 39.1%; $P < .001$). *Conclusion*: The results indicate that the SNaP toothpaste provides a greater level of efficacy in the removal of extrinsic tooth stain as compared to a regular fluoride toothpaste when used twice a day, as measured with Lobene stain index at 3 weeks and 6 weeks. *Practical Implications*: A new stannous fluoride dentifrice stabilized with nitrate and phosphates offers greater efficacy in removing extrinsic tooth stain as compared to regular fluoride toothpaste.

Stannous fluoride (SnF_2) dentifrices have shown an ability to reduce gingival inflammation, control plaque bacteria, and contribute to whole-mouth health.[1-3] However, despite being appreciated for their multifunctional benefits, SnF_2 dentifrices have been associated with a higher incidence of extrinsic tooth staining compared to sodium fluoride formulations,[4] which can effectively deliver fluoride for caries prevention without the cosmetic issues observed with stannous fluoride. Tooth color depends on both the intrinsic color of the teeth and any extrinsic stains that may develop on the tooth surface.[5] Intrinsic stains are caused during the formation of teeth. Extrinsic staining can result from smoking, consumption of colored food and drinks, aging, or exposure to metallic cations that can form colored compounds upon exposure to sulfur-containing compounds found naturally in the mouth, such as stannous ions, which is a risk associated with SnF_2 dentifrices.[6-8] To overcome this risk, different formulation methodologies for stannous fluoride have been utilized, and recent studies and advances in dentifrice formulations have demonstrated that a dentifrice containing stabilized SnF_2 offers stain prevention and removal benefits[3] in addition to prevention of caries, plaque bacteria, gingivitis, and dentin hypersensitivity.[6,7]

Smile esthetics can help motivate effective oral care routines.[9] Whitening procedures and the use of whitening products have shown to improve oral care routines and oral health.[10,11] An increasing number of oral care products focus on teeth whitening in addition to the prevention of caries and gingivitis. The whitening agents commonly used include abrasives for the mechanical removal of stains,[12,13] anti-redeposition agents to prevent the deposition of chromophores,[2,13] colorants that impart white color,[2,13] proteases for degradation of proteins,[2,13] peroxides for oxidation of organic chromophores,[13,14] and surfactants for removal of hydrophobic compounds from tooth surface.[15]

Colgate has developed a formula, a novel stannous fluoride toothpaste stabilized with nitrate and phosphates (SNaP)[16] to help prevent tooth staining and remove extrinsic stains. The new SNaP dentifrice is a promising option for effective whitening of teeth while offering protection from caries, gingivitis, and dentin hypersensitivity as well as consumer preferred flavor.[17-19]

The SNaP formula is designed to prevent stains and whiten in three ways. The stabilized formula preserves the active stannous fluoride and does not oxidize to help prevent stain occurrences; it features a polyphosphate system to prevent stain buildup without compromising the active system; and a high cleaning silica system works to safely remove extrinsic stains from enamel. In the present study, the differences between SNaP and regular fluoride toothpaste for extrinsic stain removal efficiency were measured via the Lobene stain index.[20]

Extrinsic staining can result from smoking, consumption of colored food and drinks, aging, or exposure to metallic cations that can form colored compounds upon exposure to sulfur-containing compounds found naturally in the mouth, such as stannous ions, which is a risk associated with SnF_2 dentifrices.

Material and Methods

Study Design

A phase III, randomized, single-center, two-cell, double-blind, parallel-group, and 1:1 allocation ratio clinical study was conducted to evaluate the extrinsic tooth stain removal efficacy of the SNaP (test) toothpaste (Colgate-Palmolive Co., colgatepalmolive.com) versus a negative control, commercially available fluoride toothpaste containing 0.76% sodium monofluorophosphate (Colgate-Palmolive Co.) in adults after 3 and 6 weeks of product use.

Ethics

This protocol was reviewed and approved by the U.S. Investigational Review Board, Inc. (U.S. IRB, Inc.®), 6400 SW 72 Court, Miami, Florida 33143. All study participants signed an informed consent form.

Participant Inclusion and Exclusion Criteria

Healthy patients were screened to take part in the study. Eligible participants signed an informed consent form, were in good general health, were male and female individuals aged 21 to 70 (inclusive), presented 12 scorable natural anterior teeth, had a minimum mean composite Lobene index score of 1 or greater, were available for the duration of the study, and illustrated clinical evidence of a tendency to form extrinsic stain on anterior teeth.

Participants were excluded from the study if they met any of the following conditions: presence of orthodontic bands or partial removable dentures; presence of tumors of the soft or hard tissues of the oral cavity; presence of advanced periodontal disease, characterized by purulent exudate, tooth mobility, and/or extensive loss of periodontal attachment or alveolar bone; presence of five or more carious lesions requiring immediate restorative treatment; use of antibiotics or stain-inducing medications at any time during the month prior to entry into the study; participation in any other clinical study or test panel within the month prior to entry into the study; self-reported pregnancy or breastfeeding; received a dental prophylaxis in the 4 weeks prior to the baseline examination; history of allergies to oral care/personal care consumer products or their ingredients, including hydrogen peroxide; current use of any prescription medications that might interfere with the study outcome; history of alcohol and/or drug abuse; or exposure to a tooth whitening procedure in the last 3 months.

Setting

The study period was from July 27, 2020, to September 15, 2020. The study was conducted in Trujillo Alto, San Juan Metropolitan Area, Puerto Rico.

Procedures

Qualifying participants were randomly assigned to treatment groups using a computer-generated randomization list. Qualifying individuals and all clinical study site personnel were blinded to product assignment. Toothpastes (test and control) were covered with a white adhesive label overwrap to conceal product identity. The label information on each tube consisted of a toothpaste code (ie, study group code), instructions for at-home use, and safety information, including emergency contact information. No at-home instructions were provided as to the method of brushing other than to brush twice a day for 2 minutes each time.

Data Collection

Participants were sequentially recruited at a dental office in Trujillo Alto, Puerto Rico. An experienced examiner (Dr. Arturo Elías-Boneta) collected their data from July 27, 2020, to

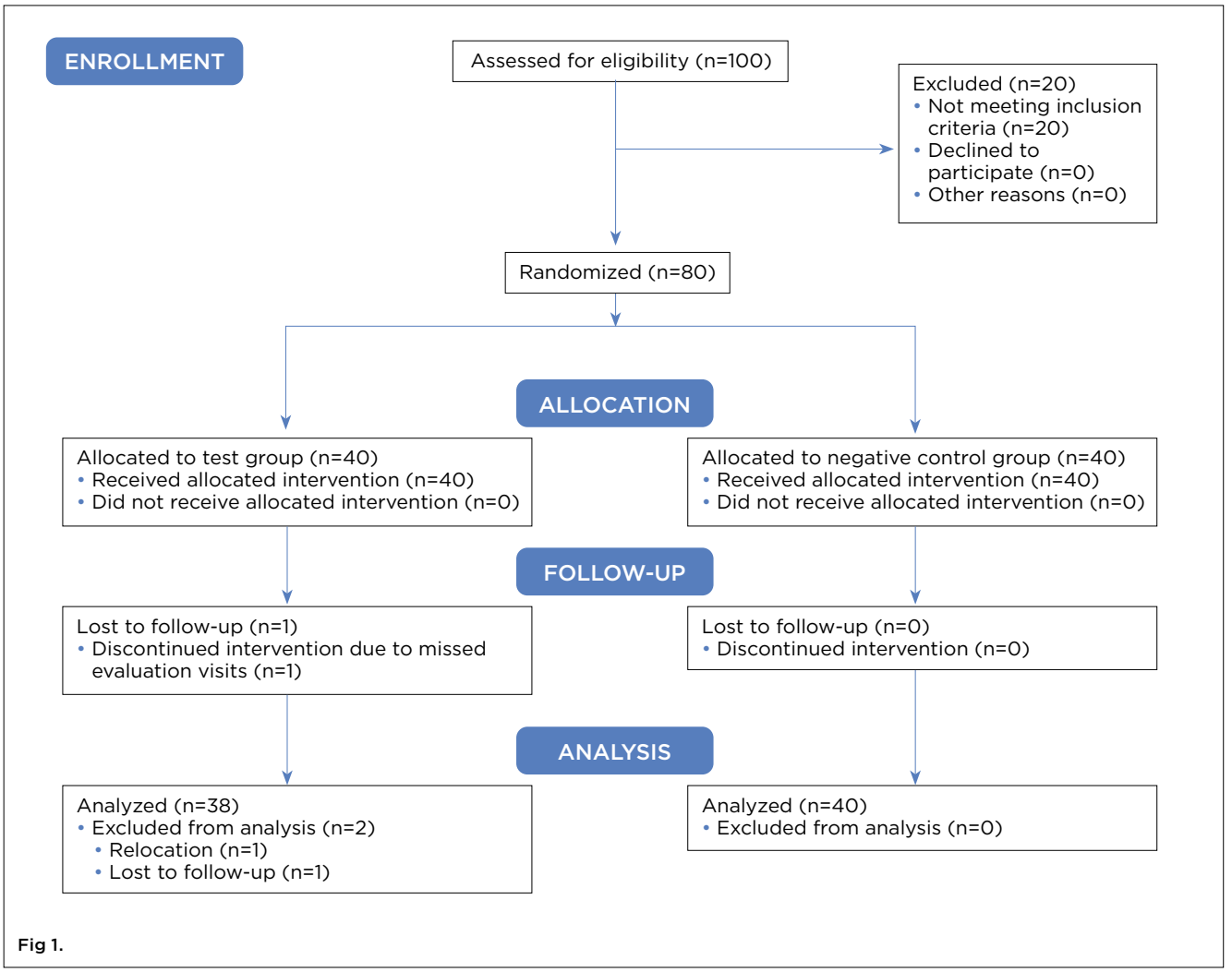

Fig 1.

Fig 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study population for the 6-week extrinsic tooth stain removal clinical study.

TABLE 1

Summary of Age and Gender for Subjects Who Completed the 6-Week Study

TREATMENT GROUP	PARTICIPANTS BY SEX[1]			AGE[1]	
	Male	Female	Total	Mean (SD)	Range
Test	14	24	38	49.13 (12.89)	24–70
Negative Control	20	20	40	49.08 (13.68)	23–70
All Treatment Groups	34	44	78	49.10 (13.22)	22–70

1 No statistically significant differences were indicated between the two treatment groups with respect to either gender (P = .241) or age (P = .985) characteristics.
SD = standard deviation

September 15, 2020. Following baseline assessments of stain and safety, participants were randomized to one of two treatment groups: (1) SNaP toothpaste or (2) a negative control, commercially available fluoride toothpaste containing 0.76% sodium monofluorophosphate. Colgate-Palmolive Co. provided the allocation list of the product codes, which was concealed in a white envelope. All office personnel and the examiner were blinded, except for the person in charge of product distribution.

Participants were instructed to brush with their assigned product twice daily for the duration of the study (6 weeks). They were

required to bring their product kit boxes to each visit, where the toothpaste tubes were weighed in a calibrated balance by the site staff to monitor and record compliance.

Sample Size

The sample size of 80 participants (40 per group) was determined based on a standard deviation for the response measure of 0.51, a significance level of α = 0.05, a 10% attrition rate, and an 80% power level. The study was powered to detect a minimal statistically significant difference between the study group means of 20%.

Assessment

The primary outcome was a mean reduction of extrinsic tooth stain via the Lobene composite stain index, stain area index, and stain intensity index after unsupervised brushing two times daily (morning and evening) for a period of 6 weeks. An experienced dental examiner blinded to product allocation codes used the Lobene stain index to evaluate extrinsic stain and soft- and hard-tissue safety at baseline, week 3, and week 6 to assess product efficacy.

Using a standard method described by Lobene,[20] each tooth was scored separately using a four-point area and intensity scale range as follows: Stain Area: 0 = no stain detected, 1 = stain up to one third of the region, 2 = stain up to two thirds of the region, and 3 = stain over more than two thirds of the region. Stain Intensity: 0 = no stain, 1 = light stain (yellow tan), 2 = moderate stain (medium brown), and 3 = heavy stain (dark brown/black).

Each participant's mean Lobene composite stain index score, comprising stain intensity and stain area, was calculated. The sum of the product (Area*Intensity) scores was divided by all sites assessed.

Statistical Analysis

Statistical analyses were performed on the Lobene composite stain index, Lobene stain area index, and Lobene stain intensity index scores. Comparisons of the treatment groups were performed using a chi-square analysis for gender and an independent t-test for age. Comparisons of the treatment groups with respect to baseline Lobene composite stain index, Lobene stain area index, and Lobene stain intensity index scores were performed using an independent t-test.

Within-treatment comparisons of the baseline versus follow-up Lobene composite stain index, Lobene stain area index, and Lobene stain intensity index scores were performed using paired t-tests. Comparisons of the treatment groups concerning baseline-adjusted Lobene composite stain index, Lobene stain area index, and Lobene stain intensity index scores at the follow-up examinations were performed using analysis of covariance (ANCOVA). All statistical tests of hypotheses were two-sided and employed a level of significance of α = 0.05.

Results

Eighty (n = 80) participants entered the clinical study, 78 individuals completed it. The recruitment period was July 27, 2020, to August 2, 2020. As stated earlier, the study period was from July 27, 2020, to September 15, 2020. The trial stopped at the end of the study period. Figure 1 shows the enrolled study population presented as a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. The reasons for not completing the study were not product related, with one participant failing to keep a study appointment, while a second one relocated. Table 1 presents a summary of the age and gender of the study population.

TABLE 2

Subject Mean Lobene Composite Stain, Stain Area, and Stain Intensity Index Scores at Baseline

INDEX	TREATMENT GROUP	n	BASELINE MEAN (SD)
Lobene Composite Stain	Test	38	1.80 (0.88)
	Negative Control	40	1.90 (0.88)
Stain Area	Test	38	1.09 (0.42)
	Negative Control	40	1.17 (0.36)
Stain Intensity	Test	38	1.20 (0.38)
	Negative Control	40	1.17 (0.41)

Participant mean Lobene composite stain, stain area, and stain intensity index scores at baseline are shown in Table 2. The per protocol population was analyzed, and observations at 3 weeks and 6 weeks are presented in Table 3 and Table 4, respectively. During the 6-week study period, 100% of the patients in the test group experienced a reduction in stain (composite stain index), while 95% in the negative control group experienced an increase in stain.

Baseline

No statistically significant difference was indicated between the two treatment groups with respect to either gender ($P = .241$) or age ($P = .985$) characteristics (Table 1). No statistically significant difference was indicated between the treatment groups with respect to the mean Lobene composite stain index ($P = .615$), Lobene stain area index ($P = .396$), and Lobene stain intensity index ($P = .817$) (Table 2).

Results at 3 Weeks

After 3 weeks of product use, the percent reductions in Lobene composite stain index scores from baseline were 24.4% for the test group and 0.5% for the negative control group (Table 3). The percent reduction from baseline observed for the test group was statistically significant ($P < .001$). However, the percent reduction from baseline observed for the negative control group was not statistically significant ($P = .813$). Relative to participants in the negative control group, participants in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 24.3% (Figure 2).

Percent reductions in Lobene stain area index and Lobene stain intensity index from baseline were also significant for the test

TABLE 3

Baseline-Adjusted Subject Mean Lobene Composite Stain, Stain Area, and Stain Intensity Index Scores at 3-Week Examination

INDEX	TREATMENT GROUP	n	ADJUSTED 3-WEEK MEAN (SE)	ADJUSTED 3-WEEK 95% CI	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISONS VS. NEGATIVE CONTROL GROUP	
					Percent Change[1]	Sig.[2]	Percent Difference[3]	Sig.[4]
Lobene Composite Stain	Test	38	1.40 (0.05)	1.31, 1.49	24.4%	$P < .001$	24.3%	$P < .001$
	Negative Control	40	1.85 (0.05)	1.76, 1.94	0.5%	$P = .813$		
Stain Area	Test	38	0.93 (0.02)	0.88, 0.98	17.4%	$P < .001$	17.7%	$P < .001$
	Negative Control	40	1.13 (0.02)	1.08, 1.18	0.9%	$P = .923$		
Stain Intensity	Test	38	0.92 (0.02)	0.87, 0.97	22.5%	$P < .001$	22.7%	$P < .001$
	Negative Control	40	1.19 (0.02)	1.15, 1.23	-0.9%	$P = .908$		

1 Percent change exhibited by the 3-week mean relative to the baseline mean. A positive value indicates a reduction in stain index scores at the 3-week examination.
2 Significance of paired t-test comparing the baseline and 3-week examinations.
3 Difference between the 3-week means expressed as a percentage of the 3-week mean for the Negative Control group. A positive value indicates a greater reduction in the stain index scores for the Test group relative to the Negative Control group.
4 Significance of the ANCOVA comparison of baseline-adjusted 3-week mean.
CI = confidence interval, SE = standard error, Sig. = significance

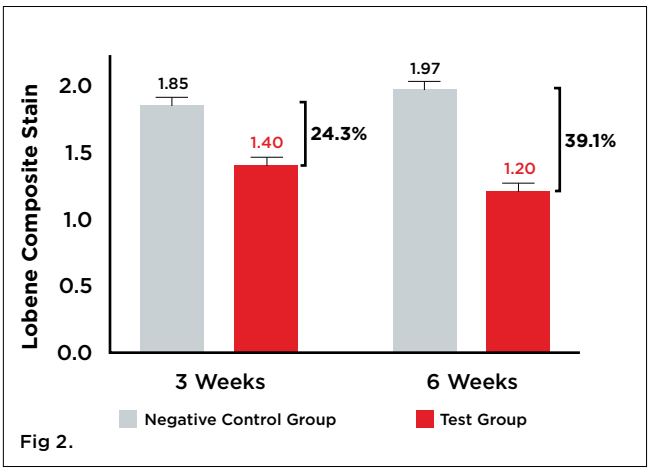

Fig 2.

Fig 2. Stain score comparison between test groups over time. Baseline-adjusted subject mean Lobene composite stain index scores and percentage change from negative control group at baseline, 3-week, and 6-week examinations.

group: 17.4% and 22.5%, respectively ($P < .001$). However, for the negative control group, changes were not statistically significant from baseline in either index (Table 3).

As measured by the Lobene stain area index, relative to participants in the negative control group, those in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 17.7% after 3 weeks of product use. As measured by the Lobene stain intensity index, relative to participants in the negative control group, individuals in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 22.7% after 3 weeks of product use.

Results at 6 Weeks

After 6 weeks of product use, the percent reductions in Lobene composite stain index scores from baseline were 35.6% for the test group, while the negative control group exhibited an increase from baseline of 5.8% (Table 4). The percent reduction from baseline observed for the test group was statistically significant ($P < .001$). However, the percent increase from baseline observed for the negative control group was not statistically significant ($P = .212$). Relative to participants in the negative control group, those in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 39.1% as measured by the Lobene composite stain index after 6 weeks of product use (Figure 2).

Percent reductions in Lobene stain area index and Lobene stain intensity index from baseline were also significant for the test group: 27.5% and 34.2%, respectively ($P < .001$). However, for the negative control group, changes were not statistically significant from baseline in either index (Table 4).

As measured by the Lobene stain area index, relative to participants in the negative control group, those in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 31.1% after 6 weeks of product use. As measured

TABLE 4

Baseline-Adjusted Subject Mean Lobene Composite Stain, Stain Area, and Stain Intensity Index Scores at 6-Week Examination

INDEX	TREATMENT GROUP	n	ADJUSTED 6-WEEK MEAN (SE)	ADJUSTED 6-WEEK 95% CI	WITHIN-TREATMENT ANALYSIS		BETWEEN-TREATMENT COMPARISONS VS. NEGATIVE CONTROL GROUP	
					Percent Change[1]	Sig.[2]	Percent Difference[3]	Sig.[4]
Lobene Composite Stain	Test	38	1.20 (0.06)	1.07, 1.33	35.6%	*P* < .001	39.1%	*P* < .001
	Negative Control	40	1.97 (0.06)	1.84, 2.10	-5.8%	*P* = .212		
Stain Area	Test	38	0.82 (0.03)	0.76, 0.88	27.5%	*P* < .001	31.1%	*P* < .001
	Negative Control	40	1.19 (0.03)	1.13, 1.25	-4.3%	*P* = .182		
Stain Intensity	Test	38	0.78 (0.03)	0.72, 0.84	34.2%	*P* < .001	35.5%	*P* < .001
	Negative Control	40	1.21 (0.03)	1.16, 1.26	-3.4%	*P* = .402		

1 Percent change exhibited by the 6-week mean relative to the baseline mean. A positive value indicates a reduction in stain index scores at the 6-week examination.
2 Significance of paired t-test comparing the baseline and 6-week examinations.
3 Difference between the 6-week means expressed as a percentage of the 6-week mean for the Negative Control group. A positive value indicates a greater reduction in the stain index scores for the Test group relative to the Negative Control group.
4 Significance of the ANCOVA comparison of baseline-adjusted 6-week mean.
CI = confidence interval, SE = standard error, Sig. = significance

by the Lobene stain intensity index, relative to participants in the negative control group, individuals in the test group exhibited a statistically significant ($P < .001$) reduction in extrinsic tooth stain of 35.5% after 6 weeks of product use.

Safety Results

No adverse effects of the hard or soft oral tissues were observed by clinical investigators or reported by the trial participants.

Discussion

This study compared the stain removal benefit of the SNaP toothpaste to regular fluoride toothpaste and demonstrated significant clinical efficacy. Participants in the SNaP test group had a statistically significant extrinsic stain reduction from baseline, 3 weeks, and 6 weeks, whereas those in the negative control group did not. Relative to participants in the negative control group, participants in the test group exhibited statistically significant ($P < .001$) reductions in extrinsic tooth stain of 24.3% and 39.1% after 3 and 6 weeks, respectively, as measured by the Lobene composite stain index, indicating a positive result for participants using SNaP.

After 6 weeks of product use, the percent reductions in Lobene composite stain index scores from baseline were 35.6% for the test group, while the negative control group exhibited an increase from baseline of 5.8%. The percent reduction from baseline observed for the test group was statistically significant.

The randomized trial was well controlled and utilized double-blinding methodology. While the inclusion/exclusion criteria outlined in the study aimed to encompass all potentially eligible participants, it is worth mentioning that the research was conducted at a single center, rather than employing a multi-center design.

Stannous fluoride has been extensively studied and is recognized for its anticaries and antigingivitis efficacy. However, patient compliance with ideal oral hygiene practices is partly driven by consumer preferences, including the desire for tooth whitening.[10,11] The formulation of SNaP is designed to prevent the oxidation of stannous ions, thereby reducing the staining typically associated with them and maintaining their efficacy. Future research should investigate the long-term whitening efficacy of SNaP to determine whether there is potential for further tooth whitening after more than 6 weeks of use. As has been measured with other whitening procedures and products,[10,11,13] future studies should also examine the relationship between the

whitening benefits of SNaP, sustained adherence to oral hygiene routines, and, consequently, improved overall oral health.

Conclusions

This study demonstrates the extrinsic stain removal efficacy of the SNaP dentifrice as measured by the Lobene stain index. This stannous fluoride formula stabilized with nitrate and phosphates provides significantly better extrinsic stain removal compared to a regular fluoride toothpaste, resulting in better whitening efficacy.

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DISCLOSURES

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DATA AVAILABILITY

The documents containing the results of the research herein described are confidential. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

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Oral health is linked to physical and mental health and your organization's bottom line.[1,2,3]

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