AMERICAN JOURNAL OF DENTISTRY

Breaking Paradigms in Toothpaste:
Unprecedented Gingival Health *and* Whitening

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CONTENTS

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Unprecedented Gingival Health *and* Whitening

A two-step stannous fluoride dentifrice and whitening gel sequence

The dramatic growth and impact of tooth whitening worldwide has raised patients' awareness of the appearance of their smile. The introduction of whitening strips in 2000 played an appreciable role, expanding access to an increasingly broad population. Some years later, the whitening concept increased to include more dentifrices developed for esthetic reasons.

At the same time, gingival health is even more important over the long-term and the prevalence of gingivitis remains high. The currently recognized gold standard chemotherapeutic treatment, chlorhexidine, has several esthetic drawbacks that limit patient compliance, and consequently effectiveness. Recognizing the widespread need for an acceptable and efficacious gingival health therapy, in tandem with patients' growing desire for whiter teeth, researchers designed a novel two-step system to meet both objectives.

This special issue of the American Journal of Dentistry highlights important research findings on gingival health, tooth whitening and safety associated with use of a unique two-step hygiene sequence: stannous fluoride dentifrice followed by hydrogen peroxide whitening gel. The research comes from diverse clinical settings.

This special issue of the American Journal of Dentistry represents one of the largest collections of clinical research on this two-stage protocol. The randomized controlled trials described herein support its significant gingival health and whitening outcomes. Such diverse testing, with respect to populations, sites and controls, provides important evidence of the merits of the two-step method. As you will see, this novel sequence shatters the long standing paradigm of therapeutic efficacy and esthetic negatives.

We hope you will find these papers interesting and educational. The Journal thanks Procter & Gamble, the manufacturer, for sponsoring this Special Issue.

Franklin García-Godoy, DDS, MS, PhD, PhD
Editor

Clinical evidence on a unique two-step stannous fluoride dentifrice and whitening gel sequence

PAUL A. SAGEL, BSChE & ROBERT W. GERLACH, DDS, MPH

ABSTRACT: Purpose: Recently, a unique two-step product was introduced that includes sequential use of a novel 0.454% stannous fluoride dentifrice followed by a 3% hydrogen peroxide whitening gel. The technology targeted advanced gingival health benefits plus esthetic benefits such as tooth whitening. The two-step sequence has unique brushing instructions to maximize the efficacy of each step; the stannous fluoride dentifrice is used for 1 minute of brushing followed by 1 minute of brushing with the hydrogen peroxide gel. This two-step sequence has been studied in numerous clinical trials over a series of years. This comprehensive program included different populations and sites, endpoints and time points, with responses measured versus different positive and negative research controls. A total of six clinical trials are reported herein. Outcomes from this research program demonstrate the significant gingival health efficacy of the two-step product, providing therapeutic efficacy comparable to chlorhexidine, and its positive impact on plaque, tooth stain and breath odor. (*Am J Dent* 2018;31:4A-6A).

CLINICAL SIGNIFICANCE: This unique dentifrice/gel sequence delivers a combination of advanced gingivitis efficacy with significant stain reduction – benefits that will positively impact oral health as well as patient compliance.

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Introduction

The majority of adults (50-90%) have gingival bleeding and inflammation.[1,2] While some individuals appear to have a greater innate susceptibility to gingivitis than others, most cases are the result of inadequate daily plaque removal.[3-6] The dental plaque biofilm is comprised of a broad array of microorganisms, and when allowed to accumulate due to suboptimal oral hygiene, the microbial population becomes increasingly virulent and provokes the inflammatory response that produces the characteristic signs of gingivitis.[5,7]

Treatment, in addition to professional and home care, may necessitate use of topical antimicrobials for plaque control. Chlorhexidine is a well-researched, long-used 'gold standard' antimicrobial for gingival health improvement.[8] As an adjunct to personal oral hygiene efforts, the favored vehicle for chlorhexidine is a 0.12% chlorhexidine gluconate mouthrinse. In a systematic review[9] of 51 clinical studies of at least 4 weeks duration, chlorhexidine mouthrinse in addition to toothbrushing was shown to provide significant reductions in plaque and gingivitis. Unfortunately, patient compliance is often a challenge due to esthetic drawbacks, namely altered taste sensation and the propensity to cause unsightly brown tooth staining requiring professional removal.[9]

Given chlorhexidine's recognition as a highly potent agent to control plaque and gingivitis, the development of a product with comparable efficacy but without chlorhexidine's objectionable taste alteration and stain proclivities would be highly desirable. Leveraging decades of formulation experience with both chemotherapeutic and cosmetic oral care products, Procter & Gamble introduced a novel technology to achieve this goal: a unique two-step system combining the therapeutic efficacy of a novel stannous fluoride dentifrice with the whitening benefits of a hydrogen peroxide gel.

Technology summary

Introduced in North America and China as Crest Pro-Health [HD][a] and Oral-B [HD],[a] respectively, this daily two-step se-

Fig. 1. Examples of the two-step sequence from the United States, Crest Pro-Health [HD], and China, Oral-B [HD].

quential system includes a 0.454% stannous fluoride dentifrice delivering caries protection and plaque/gingivitis control with a 3% hydrogen peroxide gel for tooth whitening (Fig. 1). The dentifrice and gel are separated into two sequential steps, such that the fluoride system is decoupled from the whitening technology to ensure maximum effectiveness.

Like a regular toothpaste, it is used twice daily (morning and night), however the brushing instructions are novel. First, patients brush for 1 minute with a 0.454% stannous fluoride paste (step 1). After expectorating, but not rinsing, they apply a 3% hydrogen peroxide whitening gel (step 2) to the toothbrush and continue to brush for an additional minute (Fig. 2). The total brushing time is 2 minutes, comparable to the standard recommended time. This technique is not inconvenient, as evidenced by practice reports showing favorable patient and professional satisfaction with daily usage of the system.[10]

The sequence uses stannous fluoride in step 1 for anticaries and antigingivitis properties. Stannous fluoride at 0.454% in a

Step 1: stannous fluoride paste

Therapeutic benefits, such as
- plaque
- gingivitis
- gingival bleeding

Step 2: hydrogen peroxide gel

Cosmetic benefit
- whitening

BRUSH WITH STEP 1 for 1 MINUTE   SPIT DO NOT RINSE   ADD STEP 2 / KEEP BRUSHING for 1 MINUTE   SPIT RINSE

Fig. 2. Sequential oral hygiene: 0.454% stannous fluoride paste (Step 1) and 3% hydrogen peroxide whitening gel (Step 2).

Table. Summary of clinical results for two-step stannous fluoride dentifrice and hydrogen peroxide whitening gel as reported in this special issue.

Authors	Endpoint	Duration	Control	Outcome
Gerlach & Sagel[17]	Breath Plaque Gingivitis	1 day 3 weeks 11 weeks	SMFP dentifrice (in all 3 studies)	Significant reductions in breath malodor, plaque and gingivitis favoring the two-step group versus the control.
Amini et al[18]	Gingivitis and tooth stain	3 weeks	SMFP dentifrice	The two-step sequence provided concurrent improvements in gingivitis and stain compared to the control.
Garcia-Godoy et al[19]	Gingival bleeding and tooth stain	12 weeks	0.12% chlorhexidine gluconate oral rinse plus toothbrushing with SMFP dentifrice	After prophylaxis, the two-step sequence provided comparable or superior gingivitis benefits to chlorhexidine rinse without staining.
Singh et al[20]	Plaque and saliva flow	6 weeks	SMFP dentifrice	Between-group comparisons for daytime plaque favored the two-step sequence with 41-46% improvements in plaque control. Only the two-step sequence showed increased salivary flow versus baseline.

SMFP = sodium monofluorophosphate.

dentifrice has been shown to inhibit bacterial adhesion and cohesion, reduce bacterial growth, and inhibit acid production.[11] In addition, new research suggests a role in reducing plaque pathogenicity by blocking lipopolysaccharides from inducing processes that contribute to gingivitis.[12] Like chlorhexidine, stannous fluoride dentifrices are highly effective for improving gingival health, but esthetic tradeoffs (e.g., transient extrinsic stain) were noted with early formulations.[13]

Step 2 of the novel sequence, which contains hydrogen peroxide plus an antitartar agent, provides cosmetic benefits. Hydrogen peroxide, which is commonly used in higher concentrations in professionally administered and home-based systems (e.g., whitening strips), has a lengthy history of use for tooth whitening.[14] In addition, it has been shown to be safe for long-term use and well-tolerated in dentifrices.[15] As a chemical whitening agent, hydrogen peroxide acts by disrupting stains through oxidation. Published in vitro and clinical trials have reported on the whitening actions of hydrogen peroxide-containing dentifrices compared to regular dentifrice controls, however proper formulation is critical to ensure effectiveness, typically requiring the isolation of hydrogen peroxide via packaging or other means.[16]

Clinical research

The novel brushing sequence, stannous fluoride dentifrice followed by a hydrogen peroxide whitening gel, was developed based on extensive clinical trials research studying efficacy and safety. These controlled investigations varied in study design, duration, methods, and other factors, and encompassed multiple geographies and diverse subject populations. Six clinical trials are reviewed in this *American Journal of Dentistry* special issue (Table). This supplement includes a unique review of early research on this sequence, followed by three definitive clinical trials on safety and effectiveness relative to different positive and negative experimental controls.

Gerlach & Sagel[17] report on the outcomes of three initial independent randomized and controlled clinical studies which explored the oral health benefits and safety of the new sequential two-step dentifrice and gel system. This feasibility assessment program encompassed research of varying durations and of three distinct clinical endpoints known to be favorably impacted by previous stannous fluoride dentifrice formulations: short-term research evaluating breath malodor; an intermediate-length trial of plaque control; and a longer-term investigation of gingivitis effectiveness. In each trial, the two-step 0.454% stannous fluoride dentifrice and 3% hydrogen peroxide whitening gel system was compared to a regular toothpaste[b] with twice daily usage. Collectively, these pilot trials provided evidence that the new two-step stannous fluoride dentifrice and whitening gel sequence yielded significant antimicrobial effects that were evident almost immediately (malodor), and the sequence sup-

ported a significant intermediate (plaque) and longer-term oral health effect (gingivitis). These results provided validation to proceed with additional research. Importantly, the products were well-tolerated in all of the early trials.

Amini et al[18] report on a clinical trial that specifically evaluated the primary endpoints for the two-step sequence: gingival health and whitening. The study targeted adults with both gingivitis and surface tooth stains at enrollment. Favorable responses in each endpoint were observed after 1 week of product use, and by Week 3, subjects in the two-step group averaged 39% reductions in gingival bleeding and 55% reductions in stain versus the control toothpaste.[b] Notably, 100% of the two-step users had stain reductions compared with baseline, and 97% experienced reductions in gingivitis, all without important adverse safety findings.

Garcia-Godoy et al[19] report on extended usage in a head-to-head clinical trial versus chlorhexidine[c] that models typical clinical practice conditions involving prophylaxis followed by routine monitoring for 3 months. Compared to baseline, both test groups saw significant reductions in bleeding sites at all timepoints, with the two-step group realizing directionally (4 and 12 weeks) or statistically significantly (Week 8) greater gingivitis reductions relative to the chlorhexidine group. Staining was significantly worse in the chlorhexidine group, by 94% as early as Week 4, while the two-step group experienced no significant increase in stain during the course of the trial. With chlorhexidine long considered a benchmark for gingivitis control, these study results are noteworthy in that the two-step stannous fluoride dentifrice and whitening gel provided similar or greater gingival bleeding reductions compared to the chlorhexidine positive control but without the characteristic stain associated with chlorhexidine.

Singh et al[20] share the outcomes of their 6-week clinical trial in a "so-called" vulnerable population: medication-induced xerostomia. This type of hyposalivation, which may impact as many as one-third of medicated individuals, has been reported to increase the risk of oral diseases and conditions associated with increased plaque levels.[21] Using plaque image analysis, results demonstrated that the two-step group provided 41-46% improvements in plaque control relative to the control toothpaste[b] beginning at Week 2 and continuing through Week 6. Safety outcomes were generally favorable, with the daily two-step system yielding an unexpected significant increase in salivary flow versus baseline.

Summary

The data reported in this issue demonstrate meaningful reductions in plaque, gingival bleeding and inflammation from the two-step sequence along with significant improvements in tooth stain and breath odor. Importantly, the gingivitis benefits are comparable to, or better than, the recognized gold-standard chlorhexidine. While clinicians recognize this level of gingival health improvement as foundational to oral health, the significant stain reduction offered by this product may provide the greatest motivation to many patients. Educating patients and assisting them in selecting daily-use oral hygiene products that are backed by solid and consistent research outcomes is an invaluable way to assist patients in meeting both therapeutic and cosmetic needs.

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Initial evidence of two-step dentifrice/gel sequence effects on health: Outcomes from three randomized controlled trials

ROBERT W. GERLACH, DDS, MPH & PAUL A. SAGEL, BSCHE

Abstract: Purpose: Health-related outcomes from three randomized controlled trials represented the initial research on the feasibility of novel, sequential oral hygiene with a stannous fluoride (SnF_2) dentifrice then hydrogen peroxide (H_2O_2) whitening gel. **Methods:** One crossover and two parallel clinical trials were conducted independently. Objectives varied, with individual studies assessing short, intermediate or longer-term outcomes from breath, dental plaque or gingivitis, respectively. Treatments were randomly assigned, and blinded test kits were dispensed containing either: 1) a two-step 0.454% SnF_2 dentifrice and then a 3% H_2O_2 whitening gel sequence and instructions specifying 1+1 minute sequential brushing (experimental); or 2) 0.76% sodium monofluorophosphate dentifrice (Colgate Cavity Protection) and instructions for twice daily use (control). Standard methods were used to measure efficacy (volatile sulfur compounds, plaque area coverage or gingival bleeding) and safety (clinical examination and interview), and to compare treatment responses. **Results:** Overall, 165 subjects participated in the three trials. Relative to baseline, only the experimental group exhibited significant ($P < 0.05$) improvements at initial and subsequent timepoints in each trial. Between-group comparisons showed significant ($P < 0.05$) 30-45% reductions in breath malodor (VSC), plaque (area%) and gingivitis (bleeding sites) favoring the experimental group. Adverse event occurrences were infrequent, mild in severity, and unrelated to dropout. (*Am J Dent* 2018;31:7A-12A).

CLINICAL SIGNIFICANCE: Important health-related outcomes from three initial clinical trials established the feasibility of sequential brushing with a two-step 0.454% SnF_2 dentifrice and then a 3% H_2O_2 whitening gel.

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Introduction

Dentifrices are particularly useful in the delivery of topical actives for periodontal health, in part, because of their use during daily toothbrushing, the most common oral hygiene procedure.[1] Several viable dentifrice actives have been identified, including stannous fluoride, an antimicrobial with a likely mechanism attributable to its metabolic and adherence effects on bacteria and its established retention in dental plaque.[2] The antimicrobial merits of stannous fluoride dentifrices have been recognized for decades.[3] Clinical trials[4] have demonstrated stannous fluoride effectiveness in research conducted among different populations and settings.

While stannous fluoride dentifrices can be remarkably effective, some tradeoffs were reported in earlier research.[5] Of these, esthetic limitations were the most prominent, including extrinsic dental stain that was measured/seen with longer-term use of historical stannous fluoride formulations.[6] Modern stannous fluoride dentifrices may combine potent anti-stain technologies from whitening dentifrices to mitigate esthetic consequences.[7] Notable among these is sodium hexametaphosphate, which has demonstrated significant stain prevention and removal benefits in whitening dentifrice clinical trials.[8,9] While formulation may be complex, its incorporation into stannous fluoride dentifrices has been shown to inhibit stain formation in laboratory and clinical studies.[10,11] Such formulations may have in-use characteristics (i.e: grittiness), which in turn, may affect acceptability, compliance and other outcomes among some patients.

A new product was developed to improve the in-use experience, minimize post-use esthetic consequences, and hopefully, not diminish the clinical benefits of stannous fluoride. The approach involved a novel technology (stannous fluoride dentifrice plus hydrogen peroxide whitening gel) plus novel usage (1+1 minute sequential brushing), wherein oral hygiene was separated into two consecutive steps for the explicit purpose of optimizing health and esthetic benefits. While this sequential product yielded a unique, positive brushing experience, responses were unknown, so initial clinical research was planned to assess both the health and safety implications of the new hygiene product.

Research and development for a novel technology is a complex process with temporal and resource implications. In oral care, early research has been reported to play an important role in decision making around technology development.[12] For the new stannous fluoride dentifrice plus hydrogen peroxide whitening gel sequence, this initial research consisted of randomized controlled trials to assess early, intermediate and longer term health-related responses. Breath served as a viable short-term endpoint, because of the long-standing relationships between malodor and periodontal health.[13] Plaque served as the intermediate endpoint, in part, because of the uncertain effects of novel sequential brushing on possible stannous fluoride substantivity.[14] Longer-term research over a period of months measured gingivitis, an important clinical benefit reported in previous studies[15] involving stannous fluoride dentifrices. Safety and effectiveness outcomes from this initial program were used to assess the feasibility of sequential brushing with a two-step 0.454% stannous fluoride dentifrice, and then a 3% hydrogen peroxide whitening gel.

Materials and Methods

The initial two-step oral hygiene research consisted of three randomized controlled trials that directly compared the stannous

Table 1. Study summary.

Study	Design	N	Acclimation (days)	Treatment (days)	Endpoint
Breath	Crossover	29	7	1	Volatile sulfur
Plaque	Parallel	45	7	21	Plaque area
Gingivitis	Parallel	91	0	77	Gingival bleeding

fluoride plus hydrogen peroxide sequence to a regular anti-cavity dentifrice. Study objectives and durations varied, with individual clinical trials specifically assessing breath, plaque or gingivitis over short, intermediate or longer timeframes ranging from overnight to approximately 3 months depending on the endpoint.

Despite the different objectives, several factors were common across all studies, including institutional review, informed consent, general entrance criteria, randomization, blinded test products, usage instructions and examiner-blinded evaluations. Studies differed on design, specific entrance criteria, endpoints and visits. Each clinical trial was conducted independently (in series) at different sites with different investigators, examiners and subjects, and completed over approximately a 12-month period.

Prior to study initiation, institutional review (2007094, 244-2008 and DEN05040703Exp), recruitment, and informed consent were completed, and candidate volunteers were screened for eligibility. Each of the studies targeted a generally healthy, dentate adult population without urgent dental needs or active antimicrobial treatments. Other entrance criteria were study-specific, for example, volatile sulfur, plaque or gingivitis levels at baseline, but few eligibility limits were imposed. There was one crossover (breath) and two parallel group trials, and sample sizes, acclimation and treatment duration varied (Table 1). While efficacy evaluations differed based on research objectives, safety evaluations were consistent across clinical trials, and all assessments were conducted blind to treatment assignment.

The short-term breath study was a four-period crossover with acclimation and washout periods. Usage was twice (morning and evening), with measurements 3 hours after initial use, and then overnight. The intermediate-term plaque study started with acclimation to measure baseline, treatments were assigned balancing for baseline, and overnight responses (before morning brushing) were assessed after 1 and 3 weeks of use. The longer-term gingivitis study had a baseline visit, treatment assignment, and post-treatment assessments after 5 and 11 weeks of use.

Each of the studies directly compared the two-step hygiene sequence (experimental) to regular hygiene (control) following a similar approach. Randomization was a 1:1 ratio (experimental:control) using a computer algorithm that balanced for demographics and baseline values. Subjects assigned to the experimental group received a two-step 0.454% stannous fluoride dentifrice, and then a 3% hydrogen peroxide whitening gel sequence,[a] soft manual brush (Oral-B Indicator[a]) and instructions specifying twice daily sequential 1+1 minute brushing. Subjects assigned to the control group received a marketed regular anticavity dentifrice with 0.76% sodium monofluorophosphate (Colgate Cavity Protection[b]), soft manual toothbrush (Oral-B Indicator) and instructions specifying twice daily use. For each study, test products were overlabeled and dispensed in plain white labeled kit boxes, first use was independently supervised, and subsequent use was at-home and unsupervised.

Responses were measured instrumentally and/or clinically, depending on design. For breath, volatile sulfur compounds (VSC) were measured with a calibrated, portable volatile sulfur meter (Halimeter RH17R[c]). Use of the instrument allowed quantification of hydrogen sulfide and methyl mercaptan from VSC-producing bacteria common in the oral cavity.[16] Collection followed a standard technique wherein a trained technician sampled passive breath after 2 minutes of nasal breathing, with VSC outcomes measured in ppb.[17] Overnight plaque accumulation was measured using a high resolution digital camera, polarized light and a portable microcomputer. Subjects were instructed to not brush in the morning before measurement, plaque was disclosed using a 1,240 ppm fluorescein rinse in a phosphate buffer, cheek retractors were inserted, and a single digital image was obtained. After image processing, discriminate analysis was used to ascertain disclosed plaque coverage (area%) on anterior facial tooth surfaces.[18] Gingivitis was measured at up to 168 sites (up to 28 teeth) using mild marginal stimulation with a periodontal probe, and quantified using the Löe-Silness Gingivitis Index (GI) 4-point clinical index.[19] Bleeding sites were derived from individual site scores (GI$\geq$ 2) to quantify disease severity for analysis. Safety was assessed from oral/perioral clinical examination and subject report, and adverse changes were categorized as to type, severity and causality following standard pharmaceutical research processes.

Analyses followed a priori plans using locked final databases. For the crossover breath study, VSC were analyzed on the natural logarithm scale, and mean results were back-transformed to the original ppb scale. Visits were analyzed separately using a general linear mixed model that included both random (subject) and fixed (period and treatment) effects. For the parallel group plaque and gingivitis studies, comparisons to baseline used paired-difference t-tests, while between-group comparisons used analysis of covariance with baseline as a covariate. Safety outcomes were summarized by treatment, type and severity. All statistical analyses were two-sided using a 5% significance level.

Results

The three studies enrolled a total of 165 subjects. Study populations were diverse with respect to general demographic factors, and across studies, age ranged by nearly 60 years (Table 2). In the two parallel trials, treatment groups were balanced (P> 0.54) on demographic parameters and respective efficacy endpoints (P> 0.60). Retention (82-100% by study) was high overall. While all 29 subjects in the breath study completed the first three crossover periods, five subjects missed one or more of the measurements during Period 4. In the plaque research, all 45 subjects completed the 3-week evaluation. In

Table 2. Baseline demographics by study.

Variable	Breadth (N=29)	Plaque (N=45)	Gingivitis (N=91)
Age in Years			
Mean (SD)	41.2 (8.6)	36.9 (12.8)	33.4 (11.2)
Range	25-59	19-72	20-78
Gender (N,%)			
Female	18 (62%)	34 (76%)	69 (76%)
Male	11 (38%)	11 (24%)	22 (24%)
Ethnicity (N,%)			
Asian	1 (3%)	2 (4%)	1 (1%)
Black	3 (10%)	4 (9%)	22 (24%)
Caucasian	23 (79%)	34 (76%)	36 (40%)
Hispanic	1 (3%)	9 (9%)	32 (35%)
Multiracial/Other	1 (3%)	1 (2%)	0 (0%)

the longer gingivitis study, 85 and 84 subjects were evaluated at Weeks 5 and 11, respectively.

In the breath study, baseline VSC were measured overnight (prior to brushing), while post-treatment responses were measured after 3 hours (first use) and 24 hours (overnight after second use). The crossover study showed no evidence of either carryover (P≥ 0.46) or period effects (P= 0.52). Treatments were balanced (P> 0.38) with back-transformed baseline VSC of 169 and 180 in the control and experimental groups, respectively. Treatment effects were evident relative to baseline and control at the first post-treatment timepoint, with mean VSC of 124 for the control and 82 for the two-step sequence (Fig. 1). A similar 34% between-group difference was measured at 24 hours (overnight), and at both post-treatment visits; groups differed significantly (P< 0.0001) on VSC, favoring the stannous fluoride dentifrice plus hydrogen peroxide gel sequence.

Plaque coverage was measured instrumentally on the anterior facial dentition with a focus on overnight (unbrushed) accumulation. In this inclusive study (no baseline minimum for entrance), coverage ranged from 2-37%. Mean (SD) plaque coverage was 14.9% (8.5), and groups were balanced (P> 0.73) on pre-brush levels at baseline. Overnight treatment effects were evident relative to baseline and control at Week 1 (Fig. 2). Similar responses were observed at Week 3. Overall, the two-step sequence exhibited 35-40% reductions in overnight plaque versus control, with groups differing significantly (P< 0.001) on pre-brush plaque coverage at both post-treatment timepoints.

In the gingivitis study, subjects exhibited considerable range (2-69) in bleeding sites at baseline (Fig. 3). The overall mean (SD) was 12.5 (11.8) bleeding sites, and groups did not differ significantly (P> 0.60) on gingival bleeding at baseline. Treatment effects were evident relative to baseline and control beginning at the first post-baseline visit. The ANCOVA adjusted mean (SD) changes in bleeding sites were –7.2 (11.2) and –2.6 (7.9) in the experimental and control groups, respectively. At Week 11, health improvement in the experimental group continued, while the control group did not differ significantly (P> 0.26) from baseline. Between-group comparesons at all post-baseline visits differed significantly (P< 0.02) favoring the two-step sequence.

While individual responses varied, most subjects experienced improvements in status (breath, plaque or gingivitis) during use of the stannous fluoride dentifrice plus hydrogen peroxide whitening gel sequence. This was evident across time-
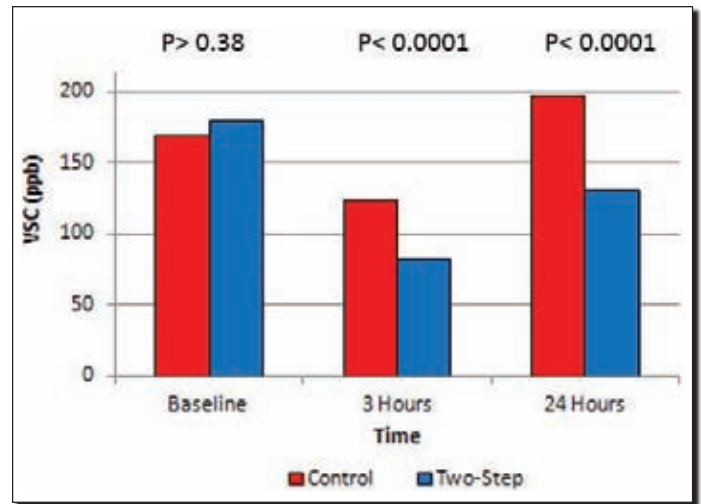
Fig. 1. Treatment comparisons for VSC by visit and group.
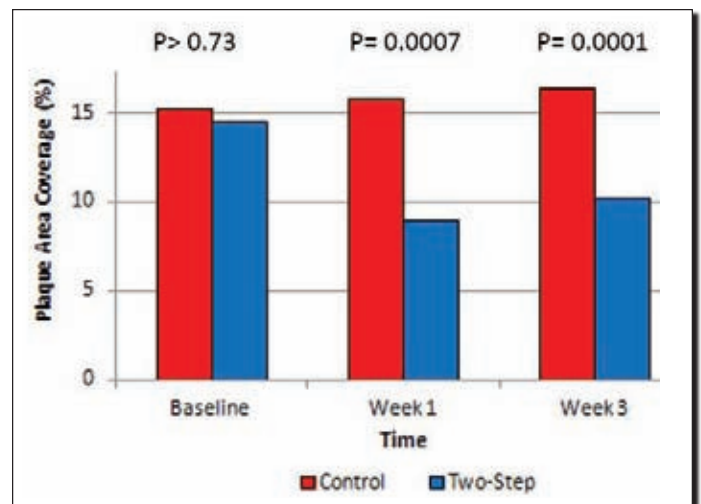
Fig. 2. Treatment comparisons for Plaque Area (%) by visit and group.
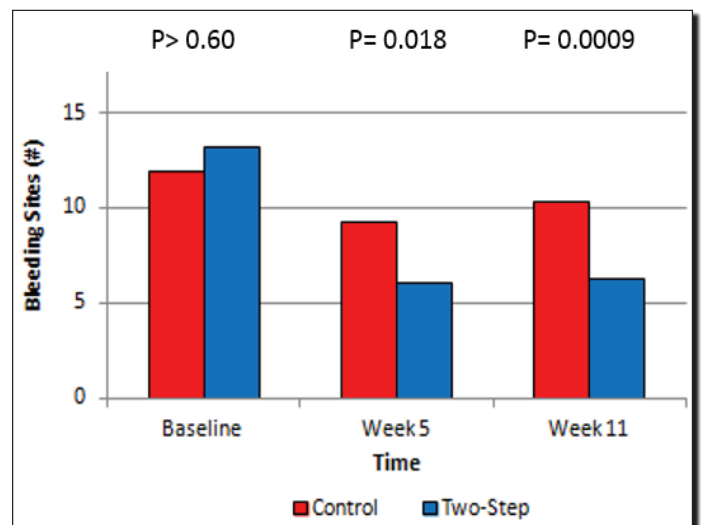
Fig. 3. Treatment comparisons for Gingival Bleeding Sites (#) by visit and group.

points and endpoints. By the endpoint, 66-97% of subjects had lower VSC, 77-83% had less overnight plaque, and 77-85% had less gingival bleeding after use of the two-step sequence. Even typical responses were impressive, as illustrated by serial images from a 21 year-old female subject in the trial, who
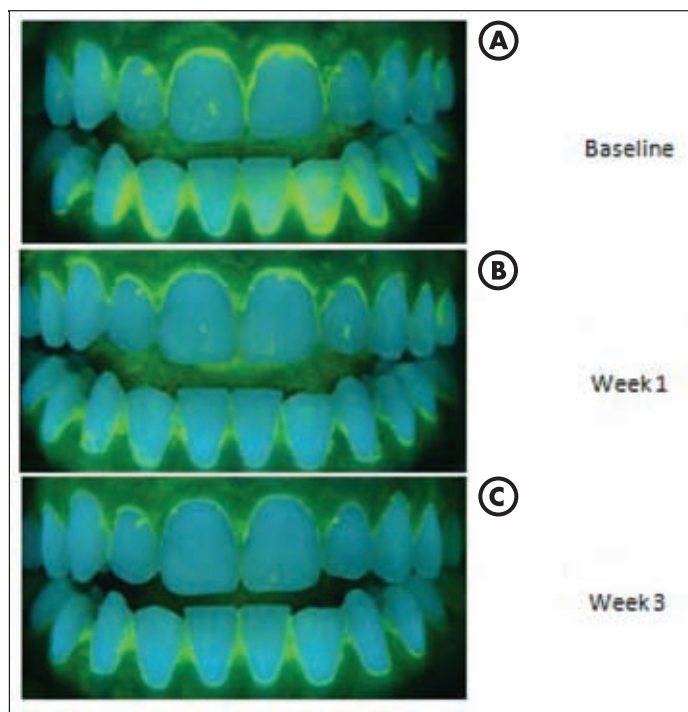
Fig. 4A-C. Overnight plaque at Baseline, and after 1 and 3 weeks use of oral hygiene sequence.

entered the research with 17.8% plaque area coverage at baseline (Figs. 4a, 4b, 4c).

These benefits were achieved without appreciable adverse experiences. Irrespective of causality, a total of six subjects had oral adverse events, three in the breath trial, two in the plaque study, and one in the gingivitis study. Only the latter of these involved the control, so by treatment, 5% of subjects using the experimental sequence and 1% of subjects using the control had an adverse event. The most common adverse event was local gingival irritation, which was mild in severity and resolved during treatment. These infrequent and minor adverse events did not contribute to any "for cause" dropouts in the three clinical trials.

Discussion

Three randomized controlled trials provided perspective at the earliest stages of the research and development process. The clinical trials were conducted in series to assess the feasibility of daily oral hygiene with a novel technology (stannous fluoride dentifrice plus hydrogen peroxide whitening gel) and novel usage (sequential 1+1 minute brushing) versus a single common control. These first studies were "pilots" without preceding evidence on population selection, study duration or other design factors characteristic of later-stage clinical trials. The initial focus was health-related outcomes measured over short, intermediate and longer time periods, and each clinical trial included multiple timepoints to assess within-study consistency. Outcomes from the exploratory research showed significant and meaningful health-related improvements for the novel oral hygiene sequence relative to both baseline and control. Effects were evident across sites and endpoints (VSC, plaque coverage and gingival bleeding) over timeframes ranging from 1 day to 11 weeks of use. Most subjects assigned to the oral hygiene sequence had measured improvements at both post-baseline visits, which were achieved without appreci-

able adverse responses. In combination, these first three clinical trials yielded perspective on adaptation, repeated use and consistency, while the different sites, methods and end-points provided evidence on the likely robustness of health-related responses and safety of this novel sequential oral hygiene.

Short-term efficacy was assessed from breath odor measured instrumentally using a portable volatile sulfur meter. Notwithstanding the obvious cosmetic benefit, breath was selected because it may represent an early health surrogate for topical antimicrobial therapies.[20] Bacterial colonies on the tongue are recognized to play an important role in malodor.[21] Use of stannous fluoride dentifrices as part of daily oral hygiene has been reported to yield significant malodor effects measured perceptually or instrumentally.[22-24] While health effects of these topical agents may take weeks-to-months to manifest, breath effects can be measured within hours-to-days, making this a viable early model to assess antimicrobial potential while limiting longer-term exposure. In the breath study, 97% of subjects had measured VSC reductions 3 hours after initial use of the stannous fluoride dentifrice plus hydrogen peroxide whitening gel sequence, suggesting ubiquitous antimicrobial efficacy with first-ever use of this novel product. Between-group comparisons showed consistent 34% reductions in overnight VSC (after second use), which were similar or greater than outcomes reported in other stannous fluoride dentifrice studies.[17,24] Importantly, the breath trial showed no evidence of adverse safety outcomes with crossover repeat use of the sequential product. The effect was also not likely attributable solely to sequential tongue brushing, which was precluded due to evidence that such targeted hygiene may impact response.[23] As such, the first breath study plausibly supported general antimicrobial effects from stannous fluoride followed by a hydrogen peroxide whitening gel without meaningful adverse effects, even with washout and rechallenge.

A plaque endpoint was selected for the intermediate duration study, in part because of the mixed clinical trials evidence on antiplaque effects with stannous fluoride delivered via toothpaste formulations.[15] Study design, formulation, esthetics and other factors may have contributed to these varied outcomes. Nonetheless, stannous fluoride has long been recognized as substantive, and this substantivity may contribute to plaque effectiveness.[14] Because of the role of brushing in plaque removal, the initial research used image analysis unambiguously to assess overnight plaque regrowth following 3 weeks of assigned daily hygiene. Results from the first plaque study provided clear evidence on response following routine use of the stannous fluoride dentifrice plus hydrogen peroxide whitening gel sequence. The experimental group exhibited a significant (P< 0.01) reduction in overnight plaque at Week 1, while routine brushing with the control dentifrice had no obvious antiplaque effects, and these responses were easily visualized via the available images. Comparing treatments, this represented approximately a 40% reduction in plaque coverage for the experimental hygiene versus control. Other negatively-controlled clinical trials have shown stannous fluoride dentifrices to have antiplaque efficacy, albeit not at the magnitude measured in this novel sequential use trial.[25-27] Responses at Weeks 1 and 3 were similar, and over three-quarters of subjects assigned to the experimental group exhi-

bited instrumentally-measured reductions in overnight plaque coverage. Of note, these consistent and impressive plaque effects provided the first evidence that the novel two-step brushing routine (stannous fluoride dentifrice immediately followed by hydrogen peroxide whitening gel) was not likely to dilute or diminish stannous fluoride activity. Rather, when combined with the minimal adverse events, outcomes from the first plaque study suggested that the sequential daily oral hygiene product may yield important health benefits.

While the breath and plaque studies assessed short-to-intermediate term, health-related outcomes using instrumental methods, the gingivitis trial was the first to measure health directly via clinical assessment (Gingivitis Index) over a period of months. Unlike plaque, systematic reviews have shown unequivocal gingivitis efficacy for stannous fluoride dentifrices used for up to 6 months.[15] Bleeding sites were selected as the endpoint of interest, and the general population recruited for the study presented with approximately 13 bleeding sites at baseline, which coincidentally, was similar to severity measured in various studies on US adults.[28,29] Gingivitis reductions of 50%+ were evident in the experimental group at both post-baseline examinations, with the majority of subjects showing improvements from baseline. Relative to the control, this represented 43% and 42% reductions in gingival bleeding at Weeks 5 and 11, respectively. That effect level substantially exceeded general criteria pertaining to meaningfulness of clinical outcomes, and in this circumstance, within the limitations of a first exploratory trial involving both a novel technology and atypical usage.[30] The consistent outcomes seen in this first health trial, without any adverse events in the experimental group, yielded the first definitive evidence of a meaningful gingivitis benefit with a sequential daily oral hygiene product.

Each of the studies had limitations, since these were the first exploratory clinical trials evaluating a new technology and usage: stannous fluoride dentifrice plus hydrogen peroxide whitening gel in sequential 1+1 minute brushing. Each of the studies was multifactorial (products and usage), so necessarily, other research would be indicated to ascertain causality. Because endpoints and timepoints differed, between-study comparisons were limited to interpretation. Nonetheless, the outcomes presented herein comprised the evidence used to assess the feasibility of developing a novel sequential daily oral hygiene product.

New product development in oral care can be quite complex, particularly for novel technologies or approaches, and early "behind the scenes" research outcomes can play an important role in progress.[31] The clinical trials reported herein represented the first evaluations of a novel daily-use approach. Outcomes demonstrated early antimicrobial activity (breath), where sequential hygiene supported, rather than diluted, a treatment effect (plaque), and a meaningful health benefit (gingivitis) was achieved without appreciable adverse events (safety). Most subjects in the sequential hygiene group experienced benefits, at a magnitude that was similar or greater than previous research on other technologies. Importantly, effects were evident across studies, times and endpoints, the latter of which showed complementary instrumental and clinical findings. Based on this research, we concluded that it was

viable to use a stannous fluoride dentifrice, followed by a hydrogen peroxide whitening gel, for routine daily oral hygiene, with expectations of achieving important health outcomes without esthetic drawbacks.

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Randomized controlled trial evaluating concurrent gingivitis and stain effects of a two-step dentifrice/gel sequence

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Abstract: Purpose: A randomized controlled trial was conducted to evaluate the clinical efficacy of a two-step dentifrice/whitening gel oral hygiene sequence on natural gingivitis and extrinsic stain. **Methods:** The population consisted of healthy adults with clinical evidence of gingivitis and extrinsic stain. Consent, demographic information and clinical measurements were collected, after which subjects were randomized to treatment. Eligible subjects were dispensed blinded test kits containing over-labeled two-step 0.454% SnF_2 dentifrice then 3% H_2O_2 whitening gel sequence or a regular 0.76% NaMFP dentifrice control (Colgate Cavity Protection), plus a regular soft manual toothbrush and instructions for use. Efficacy was assessed blind-to-treatment using the Gingivitis Bleeding Index (GBI) measured whole-mouth and the composite Lobene Stain Index (LSI) measured on the anterior dentition. Treatments were compared at Week 1 and Week 3 versus baseline for ΔGBI and ΔLSI using a two-sided 5% level of significance. **Results:** A total of 61 subjects with a mean (SD) age of 33.4 (12.0) years were enrolled. Overall baseline means (SD) were 0.16 (0.05) for GBI and 1.30 (0.94) for LSI. After 1 week, only the two-step 0.454% SnF_2 dentifrice then 3% H_2O_2 whitening gel sequence demonstrated significant (P< 0.0001) reductions in both gingivitis and stain. Adjusted means for the changes with the dentifrice/gel sequence and control were –0.055 and –0.001 for ΔGBI, and –0.619 and –0.095 for ΔLSI, with groups differing significantly (P< 0.0001) on gingivitis and stain improvement. Outcomes at Week 3 were generally similar, with groups differing on bleeding and stain. Treatments were generally well-tolerated. (*Am J Dent* 2018;31:13A-17A).

Clinical significance: In a randomized controlled trial, use of a two-step 0.454% SnF_2 dentifrice then 3% H_2O_2 whitening gel sequence yielded concurrent improvements in gingivitis and stain compared to regular oral hygiene.

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Introduction

The role of daily oral hygiene in plaque control and cleaning is well-established. Unfortunately, home care techniques are often imperfect, and the visible effects of poor plaque control on oral health have been recognized for at least 50 years as the rapid onset and increased severity of local gingivitis.[1] Prevalence is common despite routine home care. Approximately one-half of US adults have gingival bleeding, and some populations exhibit disproportionately higher gingivitis levels.[2,3]

At-home care can be enhanced by the use of topical antimicrobials, and extensive research has demonstrated anti-gingivitis effects for up to 6 months with at-home daily use.[4,5] Despite this effectiveness, there is long-standing recognition of tradeoffs between health and esthetics with topical antimicrobial treatments, the most common of which is development of extrinsic tooth stain. Research[5-10] conducted over several decades has shown the potential for different rinses and dentifrices with therapeutic actives such as cetylpyridinium chloride, stannous fluoride, essential oils and others to contribute to tooth staining. Various mechanisms have been suggested, depending on the antimicrobial agent in question.[11] A multi-factorial etiology including contributions from dietary chromogens is likely, as staining potential varies widely between individuals.

Chlorhexidine plausibly represents the archetype of 'health versus esthetics' outcomes. Developed as a long-term adjunct for daily oral hygiene, a 0.12% chlorhexidine digluconate rinse has been shown in definitive clinical trials research to significantly reduce plaque and gingivitis over a 6-month period between dental visits.[12] Other effects have been explored, including use of chlorhexidine for microbial control during implant placement, post-surgical periodontal maintenance, and prevention of alveolar osteitis from third molar surgery.[13-15] Despite its original indication for between-recall gingivitis, chlorhexidine is primarily confined to short-term use because of an adverse event profile that includes calculus formation and noteworthy staining, the latter of which may develop within a few days or weeks of use.[16] One illustration of the potential for tooth staining with chlorhexidine is its use in tooth stain induction research models, where antimicrobial-related stain is rapidly formed, in order to study the relative effectiveness of whitening dentifrices on stain prevention and removal.[17,18] While treatment is generally uncomplicated, and antimicrobial staining can be typically managed or prevented using various whitening dentifrices or routine prophylaxis, visible side effects like staining remain an ever-present challenge to compliance, and therefore, to the favorable health responses that may be achieved with many common actives.

Is there a technology with the usage benefits from daily oral hygiene that combines effectiveness and acceptable esthetics (i.e: without extrinsic dental stain)? This question was specifically evaluated using a novel technology that separated oral hygiene into two consecutive steps for the explicit purpose of optimizing health and esthetic benefits. The first step involves brushing for 1 minute with a 0.454% stannous fluoride dentifrice, while the second step follows with brushing for 1 minute with a 3% hydrogen peroxide whitening gel. This easy-to-use sequence maintains brushing time at approximately 2 minutes, with separate, consecutive therapeutic and esthetic steps. Clinical research was conducted to simply assess whether this two-

step sequential oral hygiene yielded concurrent health and esthetic outcomes.

Materials and Methods

A randomized controlled trial was conducted to evaluate the effects of a two-step dentifrice/whitening gel oral hygiene sequence on oral health and esthetics. The research, which was conducted with appropriate human subjects review (U.S.IRB 2013SRG/01) following written informed consent, targeted generally healthy adult volunteers with clinical evidence of naturally-occurring gingivitis and extrinsic stain. Inclusion was limited to adults (18+ years old) with at least six natural anterior teeth, plus evidence of visible extrinsic stain on four anterior teeth, and gingivitis-associated bleeding at 15 sites, while subjects were excluded due to pregnancy, fixed ortho-dontic appliances or recent antibiotic use. There were three study visits over a 3-week period. After baseline measure-ments, eligible subjects were randomly assigned to treatment and test products were dispensed. Clinical examinations were conducted after 1 and 3 weeks of assigned product usage to assess both absolute and comparative safety and efficacy of the two-step system and control.

There were two daily oral hygiene treatment groups, the two-step hygiene sequence experimental group and a regular control. Test products were randomly assigned by computer algorithm on a 1:1 ratio in blocks of four, balancing for gingivi-tis ($\leq$ 24, > 24 bleeding sites) and stain ($\leq$ 0.6, > 0.6 composite LSI). The experimental group received a two-step sequence: 0.454% stannous fluoride dentifrice, then 3% hydrogen peroxide whitening gel,[a] while the control group received a marketed regular anticavity dentifrice[b] with 0.76% sodium monofluoro-phosphate. These test products were overlabeled to disguise their identities, and since the treatments differed in appearance (two tubes versus one), test products were dispensed in a plain white labeled kit box with a soft manual brush (Oral-B Indicator[a]) and instructions. Instructions specified twice daily use either as sequential 1+1 minute brushing for the experimental group or regular hygiene practices for the control group. First use was independently supervised to maintain blinding, and subsequent use was at-home and unsupervised.

The study assessed concurrent effects of hygiene on oral health, esthetics and safety/tolerability. Each outcome was measured at each visit by a single treatment-blinded examiner using long-standing clinical indices to assess health and esthetics. For oral health, the primary endpoint was gingival bleeding, which was assessed across the whole mouth and quantified using the Gingival Bleeding Index (GBI).[19] This method used mild provocation of the gingival crevice with a periodontal probe at 2 mm depth passed gently circum-ferentially around each tooth at approximately a 60° angle. After 30 seconds, each tooth site was assessed and bleeding was quantified with respect to absence/presence and severity using a standard 3-point scale. For oral esthetics, the primary endpoint was visible extrinsic tooth stain, which was assessed on the facial and lingual surfaces of the anterior teeth and quantified using the Lobene Stain Index (LSI).[20] With this method, each tooth was divided into gingival and body regions, the former of which represented approximately a 2 mm band along the margin, while the latter represented the remaining tooth surface. Within each region, extrinsic stain was assessed

Table 1. Baseline demographics by group and overall.

Variable	Experimental group (N=30)	Control group (N=31)	Overall (N=61)	P-value
Age in years				
Mean (SD)	33.6 (12.69)	33.3 (11.39)	33.4 (11.95)	0.92
Range	18-64	20-66	18-66	
Gender (N,%)				
Female	17 (57%)	18 (58%)	35 (57%)	0.99
Male	13 (43%)	13 (42%)	26 (43%)	
Ethnicity (N,%)				
Asian	3 (10%)	4 (13%)	7 (11%)	0.43
Black	4 (13%)	9 (29%)	13 (21%)	
Caucasian	10 (33%)	10 (32%)	20 (33%)	
Hispanic	6 (20%)	4 (13%)	10 (16%)	
Multiracial/Other	7 (23%)	4 (13%)	11 (18%)	

Table 2. Baseline gingivitis and stain by group and overall.

Variable	Experimental group (N=30)	Control group (N=31)	Overall (N=61)	P-value
Gingivitis (GBI)				
Mean (SD)	0.17 (0.06)	0.15 (0.04)	0.16 (0.05)	0.22
Bleeding sites (SD)	24.6 (7.31)	23.5 (6.00)	24.1 (6.64)	0.52
Tooth Stain (LSI)				
Composite	1.26 (0.73)	1.34 (1.12)	1.30 (0.94)	0.75
Area	0.59 (0.29)	0.57 (0.38)	0.58 (0.34)	0.89
Intensity	1.08 (0.62)	1.05 (0.62)	1.06 (0.62)	0.82

with respect to coverage and intensity and quantified using standard 4-point scales. Safety was assessed by oral examina-tion, and any oral adverse events were coded by severity and causality using standard pharmaceutical research practices.

The principal effectiveness (GBI & LSI) and safety (oral examination & adverse events) data, demographics, and any other measurements were collected by direct entry using a portable computer and transmitted blind to treatment for evaluability and analysis. After evaluability, the database was locked, treatment was assigned, and analysis was conducted following an a priori plan. In brief, demographic data were summarized by treatment and overall. For oral health, whole mouth GBI scores were derived by subject and visit by summing the gradable site-level GBI scores and dividing by the number of gradable sites. For esthetics, mean LSI scores were derived in a similar fashion. Comparisons to baseline were conducted using paired t-tests. Treatment groups were com-pared using analysis of covariance with baseline, treatment and interactions. Safety data were summarized by treatment and overall. Secondary analyses of the subject-level relationships between health and esthetic outcomes were investigated using Pearson correlations. All comparisons were two-sided with a significance level of 5%.

Results

After screening, informed consent and baseline measure-ments were obtained from 61 adults. The study population exhibited appreciable diversity in gender, ethnicity and age, the latter of which ranged from 18-66 years (Table 1). For gingivitis, the mean (SD) GBI was 0.16 (0.05), with a mean (SD) 24.1 (6.6) bleeding sites ranging from 15-47. For stain, the mean (SD) LSI composite was 1.3 (0.94), with some subjects exhibiting extensive dark staining. After baseline, randomiza-

Table 3. Week 1 changes in gingivitis and stain levels by group.

Variable	Experimental group N=30	P value	Control group N=31	P-value
Gingivitis (GBI)				
Mean (SD)	-0.06 (0.04)	<0.0001	0.00 (0.03)	0.83
Bleeding Sites (SD)	-8.30 (5.44)	<0.0001	-0.19 (4.69)	0.82
Tooth Stain (LSI)				
Composite	-0.60 (0.58)	<0.0001	-0.09 (0.46)	0.003
Area	-0.26 (0.26)	<0.0001	-0.05 (0.08)	<0.002
Intensity	-0.47 (0.47)	<0.0001	-0.05 (0.14)	<0.04

Table 4. Week 3 changes in gingivitis and stain levels by group.

Variable	Experimental group N=30	P value	Control group N=31	P-value
Gingivitis (GBI)				
Mean (SD)	-0.08 (0.05)	<0.0001	-0.01 (0.05)	0.08
Bleeding Sites (SD)	-10.93 (5.79)	<0.0001	-2.10 (5.70)	0.05
Tooth Stain (LSI)				
Composite	-0.82 (0.61)	<0.0001	-0.31 (0.46)	0.0009
Area	-0.33 (0.17)	<0.0001	-0.13 (0.17)	<0.0003
Intensity	-0.66 (0.48)	<0.0001	-0.16 (0.14)	0.007

Fig. 1. Comparative improvement (%) for SnF_2/H_2O_2 Sequence vs Control.

Fig. 2. Change in GBI and LSI by Subject and Event, SnF_2/H_2O_2 Sequence.

tion yielded 30 and 31 subjects assigned to the experimental and control groups, respectively, with groups balanced (P> 0.20) on gingivitis and stain (Table 2). One subject in the experimental group voluntarily withdrew after Week 1. All other subjects completed all visits and were included in the analyses.

Relative to baseline, the two-step group showed improvements in both health and esthetics at Week 1, as evidenced by significant reductions (P< 0.0001) in GBI, bleeding sites, and LSI, with the latter evident for the composite and individual measures of stain area and stain intensity. In contrast, the control group showed no significant (P≥ 0.82) changes in GBI or bleeding sites at Week 1, but did show significant (P< 0.04) reductions in LSI composite, area and intensity. Results were generally consistent at Week 3 (Tables 3, 4).

Comparing treatments, the Week 1 adjusted bleeding sites means (SE) were 15.9 (0.91) and 23.9 (0.86) in the experimental and control groups, respectively. The Week 1 adjusted LSI composite means (SE) were 0.68 (0.08) in the two-step group compared to 1.2 (0.03) in the control. Groups differed significantly (P< 0.0001) on bleeding sites and stain favoring the two-step oral hygiene sequence. At Week 3, the adjusted bleeding site means (SE) were 13.3 (0.96) for the experimental group compared to 21.8 (1.04) for the control, with groups differing significantly (P< 0.0001). Stain showed similar responses, with groups differing significantly (P< 0.0001) on stain removal throughout the 3-week period. For the principal health and esthetic endpoints, this represented 33-39% reductions in bleeding and 44-55% reductions in stain for the stannous fluoride dentifrice plus hydrogen peroxide whitening gel sequence versus control, depending on endpoint and visit (Fig. 1).

There were four adverse events (one per subject), each of which was in the experimental group, and coded as hyperesthesia (two subjects) or gingival irritation (two subjects). Of these, three events were reported during the subject interview, and one (gingival irritation) was observed on examination. Each of these was considered mild in severity, and resolved without discontinuation of assigned treatment.
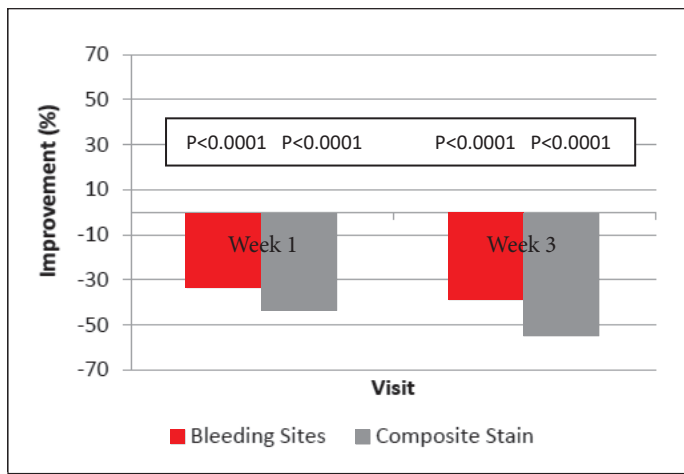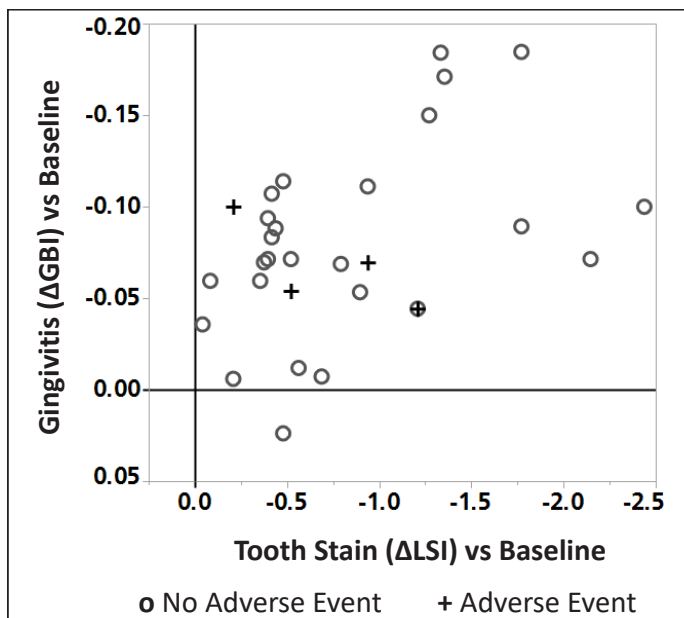
Secondary analysis specifically assessed the relationships between the health/esthetic outcomes and the possible impact of adverse events on effectiveness. This analysis compared the Week 3 GBI and LSI composite scores for all subjects (N=30) assigned to the stannous fluoride dentifrice plus hydrogen peroxide whitening gel group. Relative to baseline, there were highly significant (P< 0.0001) reductions in gingivitis (47.1%) and stain (65.1%) after 3 weeks' BID use. Gingivitis and stain responses were correlated (r= 0.42). Overall, 97% of subjects exhibited improvement in gingivitis, 100% exhibited improvements in stain, and 13% had one adverse event. Subjects with adverse events had generally similar mean responses to those without adverse events (Fig. 2). Only one subject (3%) failed to exhibit improvement in both health and esthetics after 3 weeks use of the stannous fluoride dentifrice plus hydrogen peroxide hygiene whitening gel sequence.

Discussion

This new randomized controlled trial directly assessed concurrent health and esthetic outcomes with two-step daily

oral hygiene, as these endpoints were the primary intention behind development of the novel stannous fluoride dentifrice plus hydrogen peroxide gel technology. With this focus, the target population was adult volunteers with evidence of visible gingivitis and extrinsic tooth stain. Eligible subjects were randomly assigned test products, and the health and esthetic outcomes were measured clinically after 1 week to assess initial response and after 3 weeks to assess durability. Results from the research demonstrated that the two-step sequence yielded significant (P< 0.0001) concurrent improvements in both gingivitis and stain beginning at the first post-treatment visit (Week 1), and these results were sustained through the second visit (Week 3). Relative to baseline, this represented 35-65% reductions in gingival bleeding and overall tooth stain, with benefits evident across methods and timepoints.

The clinical study compared daily oral hygiene with the novel two-step sequence head-to-head versus a normal hygiene control. Groups were dispensed blinded test products with two or one steps, along with a common manual brush and specific marketed instructions for use. Both groups had measured improvements in gingivitis and stain, though treatments differed with respect to response. At Week 1, use of the two-step sequence yielded a mean 8.30 bleeding site reduction, compared to 0.19 for the control. Stain response was generally similar with a mean reduction of 0.60 for the two-step group, compared to 0.09 for the control. Overall, this represented at least a 6-fold initial concurrent improvement in gingivitis and stain for the population studied in this research. Initial differences persisted, and between-group comparisons demonstrated significant (P< 0.0001) reductions in both gingivitis and stain for the stannous fluoride plus hydrogen peroxide group throughout the clinical trial.

There were three endpoints: health measured as gingivitis, esthetics measured as extrinsic stain, and safety measured via adverse events during treatment. Interestingly, each of these endpoints may have brushing implicated in the nominal etiology. Gingivitis is the most obvious of these, with its widespread recognition as an inflammatory response to plaque accumulation usually as a result of inadequate oral hygiene. One long standing research approach (the so-called "experimental gingivitis" model) involves suspension of tooth brushing and subsequent disease induction a few days thereafter.[1] Extrinsic stain has a potentially more complex etiology that includes behavior and diet, tooth brushing and other factors.[21] Like gingivitis, extrinsic stain accumulation can be accelerated by suspending tooth brushing, along with concurrent use of agents like tea or chlorhexidine.[18] Safety is the least obvious endpoint with respect to brushing etiology. Research has implicated tooth brushing in gingival abrasion, though clinical manifestations may be sufficiently modest to necessitate use of disclosing solutions for detection and quantification.[22] Other research[23,24] has suggested a role of brushing frequency, though outcomes are generally ambivalent.

The research has some limitations, given the objective of measuring concurrent gingivitis and stain. Both outcomes are commonly seen in dental practice and/or via population surveys. In research, more than one-half the US population exhibits gingivitis.[2,25] Numerous options exist for quantifying gingivitis, and this research focused on marginal bleeding

(measured using GBI) because it has previously been recognized as a simple and reliable indicator of health/disease.[26] In addition to its clinical utility, the GBI index allowed easy quantification of bleeding site numbers (GBI> 0), an important metric of disease extent and severity. Extrinsic stain development has been less studied in population surveys, and its occurrence is less well known. In this study, extrinsic stain was measured on anterior tooth surfaces using a common clinical trials method.[27,28] While meaningful, this approach differed from the "whole mouth" measurements made for gingivitis, so further research would be indicated to assess concurrent stain reduction in the posterior dentition. Other endpoints may have yielded different outcomes. More importantly, the research was confined to subjects with both gingivitis and extrinsic stain. While these are plausibly concurrent conditions, inference from this specific research may be limited to those individuals presenting with both gingival disease and tooth stain. Finally, this research used a multivariable design under labeled usage conditions pertinent to patient care, and other single-variable studies may be indicated to ascertain causality.

Outcomes from this new research support use of the novel two-step dentifrice/gel sequence for daily oral hygiene, given the important immediate and durable health benefits with the absence of stain formation. In fact, there was actual stain reduction relative to both baseline and control evident after just 1 week of at-home use of the sequence. Adverse events with the two-step hygiene were uncommon and minor, and did not negatively affect individual health or esthetic responses. Long term health and well-being implications with two-step oral hygiene are unknown, but potentially important. For the former, consistent gingival bleeding has been implicated with other adverse outcomes, including loss of periodontal attachment, and conceivably, tooth loss.[29] For the latter, new research[30] suggests that treatment may improve oral health quality of life. While such research awaits, daily at-home use of the stannous fluoride plus hydrogen peroxide sequence yielded early, meaningful health effects without esthetic tradeoffs.

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Post-prophylaxis gingivitis prevention with two-step stannous fluoride dentifrice plus whitening gel sequence or chlorhexidine gluconate mouthrinse

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ABSTRACT: Purpose: To assess use of a two-step dentifrice/gel sequence versus chlorhexidine gluconate mouthrinse on gingivitis prevention after dental prophylaxis. Methods: A 12-week, randomized controlled trial was conducted to compare the effectiveness and safety of a two-step dentifrice/gel sequence to a positive control in healthy adults with established gingivitis. After informed consent, gingivitis and stain levels were assessed by clinical examination. Eligible subjects received a dental prophylaxis and were randomly assigned to twice daily unsupervised use of either (1) two-step oral hygiene sequence: 0.454% stannous fluoride dentifrice followed by 3.0% hydrogen peroxide whitening gel for the test group; or (2) 0.12% chlorhexidine gluconate oral rinse and 0.76% sodium monofluorophosphate dentifrice for the control group. Clinical measurements of gingivitis bleeding sites and tooth stain area/intensity were collected after 4, 8 and 12 weeks use, while safety was assessed via clinical examination and oral status interview of the subjects. Results: A total of 44 subjects were enrolled and 35 completed the 12-week study. At baseline, bleeding sites ranged from 10-33. After prophylaxis and assigned treatment, both groups exhibited significant ($P \leq 0.0001$) reductions in bleeding sites. Responses were directionally better in the two-step sequence at all post-baseline timepoints, with groups differing significantly ($P < 0.05$) at Week 8. Tooth stain measurements demonstrated that the two-step dentifrice/gel sequence did not contribute to any significant ($P > 0.13$) stain accumulation. In contrast, stain accumulation was evident ($P < 0.003$) in the chlorhexidine group beginning at the Week 4 visit. Adverse events were more common in the positive control, and contributed to early termination. (*Am J Dent* 2018;31:18A-23A).

CLINICAL SIGNIFICANCE: Twice daily use of a two-step stannous fluoride dentifrice and peroxide whitening gel sequence after prophylaxis provided comparable or superior gingivitis benefits to chlorhexidine gluconate rinse without the concomitant side effect of staining.

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Introduction

Gingivitis, an inflammation of the gingival tissues without loss of connective tissue attachment, is a highly prevalent oral health disease.[1] It is reported to affect four out of five adults globally,[2] and approximately 90% of American adults have signs of gingivitis of at least mild severity.[3]

Dental plaque plays a prominent etiological role in gingivitis onset.[4] The resulting localized inflammatory response manifests as gingival redness, swelling, and bleeding.[5] Dental prophylaxis and daily oral hygiene represent the most common approaches to treat and prevent gingivitis. Both professional and at-home treatment may be supplemented by the use of antimicrobial agents, including triclosan, stannous fluoride, essential oils or cetylpyridinium chloride, which have been reported to yield antigingivitis effects when delivered via dentifrices or mouthrinses.[6-8]

One of the most recognized antimicrobials is chlorhexidine, which has been studied for at least 50 years and has proven effectiveness. One of the most common approaches is as a prescription-based 0.12% chlorhexidine gluconate rinse. Clinical research has established effectiveness to improve and maintain gingival health following clinical intervention.[9,10] A systematic review[11] of six clinical trials of more than 6 months duration found a weighted mean plaque index percentage decrease of 40% and a gingivitis index reduction of 28% with chlorhexidine. Another systematic review[12] of 18 gingivitis prevention trials of at least 4 weeks duration reported that chlor-

hexidine provided 33% greater plaque reduction and 27% greater gingivitis prevention than did control agents. As such, chlorhexidine rinses are widely recognized as the "gold standard" for care, however their use is associated with undesirable side effects that can impact patient compliance and clinical effectiveness. While some adverse events are uncommon, two are prominent: extrinsic tooth staining and altered taste sensation. Extrinsic tooth staining has been shown to manifest after as little as 3 days of chlorhexidine usage in digital colorimeter research.[13] Altered taste sensation has been reported, especially with longer term use.[14] Each of these can substantially hinder patient compliance, and thereby minimize the utility of chlorhexidine, particularly longer-term use.[15,16]

Plaque accumulation after prophylaxis and subsequent gingivitis has contributed to interest in oral care products with the effectiveness of chlorhexidine but without the problematic staining. One such product is a novel daily two-step dentifrice/gel sequence. Step 1 is a dentifrice containing stannous fluoride, a well-studied antimicrobial agent with anticaries, antiplaque, antigingivitis, and sensitivity reduction efficacy.[17-22] Step 2 utilizes a hydrogen peroxide whitening gel. In the current 12-week investigation, this novel, over-the-counter, two-step 0.454% stannous fluoride dentifrice/3% hydrogen peroxide whitening gel sequence, (marketed as Crest Pro-Health [HD][a] or Oral-B [HD],[a] depending on the region), was compared to a control regimen of a 0.12% chlorhexidine mouthrinse and 0.76%

Table 1. Baseline subject characteristics (randomized subjects).

	Two-Step Sequence	Chlorhexidine	Overall	P-value
Demographic parameter	N=21	N=23	N=44	
Mean Age, years (SD)	34.3 (10.53)	38.0 (12.49)	36.3 (11.62)	0.2892[a]
Age Range, years	19-62	20-65	19-65	
Female (n, %)	12 (57%)	14 (61%)	26 (59%)	1.0000[b]
Male (n, %)	9 (43%)	9 (39%)	18 (41%)	
Asian Indian (n, %)	1 (5%)	0 (0%)	1 (2%)	0.3434[b]
Asian Oriental (n, %)	1 (5%)	1 (4%)	2 (5%)	
Black (n, %)	5 (24%)	7 (30%)	12 (27%)	
Caucasian (n, %)	10 (48%)	6 (26%)	16 (36%)	
Hispanic (n, %)	4 (19%)	9 (39%)	13 (30%)	
Gingivitis/Stain parameters				
Number of Bleeding sites (mean, SD)	16.57 (6.01)	17.13 (7.21)	16.86 (6.60)	0.7826[a]
Lobene Composite Stain Score (mean, SD)	0.11 (0.27)	0.16 (0.40)	0.14 (0.34)	0.5952[a]

SD = standard deviation; n = number of subjects; % = percentage.
[a] Two-sided ANOVA for the treatment comparison.
[b] Two-sided Fisher's exact test for the treatment comparison.

sodium monofluorophosphate dentifrice in order to assess their relative effectiveness for gingival health maintenance and esthetics, in subjects with pre-existing mild-to-moderate gingivitis.

Materials and Methods

A 12-week randomized controlled clinical trial was conducted to compare post-prophylaxis effectiveness and safety of an experimental oral hygiene regimen versus a positive control. Institutional review and approval was obtained from Nova Southeastern University Institutional Review Board (NSUIRB No. 06301425Exp.). The study was conducted in compliance with the International Conference on Harmonization's Good Clinical Practice Consolidated Guidelines. All subjects provided written, informed consent. Eligibility was limited to generally healthy adults 18 years of age or older with at least 16 gradable teeth, and presenting with a minimum of 10 bleeding sites at baseline. Subjects with severe periodontal disease, active treatment for periodontitis, fixed facial or lingual orthodontic appliances, or antibiotic use within 2 weeks of baseline were excluded. Up to 50 subjects were targeted for enrollment based on non-inferiority testing for the experimental group versus control, and the 12-week time frame was selected to assess the longer-term durability of health effects after prophylaxis.

At baseline, a thorough medical history was obtained for each subject and a comprehensive clinical examination of the oral and perioral regions, including the hard and soft tissues, was conducted. Following the oral examination, extrinsic tooth stain was measured using the Lobene Stain Index and gingivitis was measured using the Löe-Silness Gingivitis Index.[23,24] Subjects who met all entrance criteria received a thorough dental prophylaxis from a dental hygienist within approximately 7-10 days of baseline. Subjects were then stratified by age, gender, number of bleeding sites, and stain levels, and assigned randomly to either the test group (twice-daily brushing with 0.454% stannous fluoride dentifrice followed by 3.0% hydrogen peroxide whitening gel[a]) or to the positive-control group (twice-daily brushing with 0.76% sodium monofluorophosphate dentifrice, Colgate Cavity Protection,[b] and twice-

daily rinsing with 0.12% chlorhexidine oral rinse, Peridex[c]). Both groups were provided with a soft, flat-trim manual toothbrush (Oral-B Indicator[a]) and a timer in test kit boxes that were identical in appearance for blinding purposes. All subjects were instructed to use the study products in place of their usual oral hygiene products for the duration of the study.

Those assigned to the test group were instructed to brush twice daily. First, they were to brush for 1 minute with the Step 1 stannous fluoride dentifrice and then to expectorate without rinsing. They were instructed to next brush with the Step 2 hydrogen peroxide gel for 1 additional minute. After brushing with the Step 2 gel, subjects were to expectorate, and then rinse with tap water. Subjects assigned to the control group were instructed to brush in their customary manner twice daily with the provided sodium monofluorophosphate dentifrice, followed by rinsing with 15 mL of undiluted chlorhexidine oral rinse for 30 seconds using the provided dosing cups.

Subjects were recalled after 4, 8 and 12 weeks of unsupervised product use. At each visit, continuance criteria were assessed, safety was assessed by interview and examination, and gingivitis and tooth stain were measured by clinical examination by a trained, qualified dentist who was blind to treatment assignment. Gingivitis was measured at up to 168 sites (maximum 28 teeth) using mild marginal stimulation with a periodontal probe and recorded using the Löe-Silness Gingivitis Index ranging from 0 to 3.[24] Bleeding sites were derived from individual site scores (GI≥ 2) to yield a practice-relevant dichotomous endpoint of gingival health.

The primary endpoint for oral esthetics was visible extrinsic tooth stain, evaluated on the facial and lingual surfaces of the 12 gradable maxillary and mandibular anterior teeth and quantified using the Lobene Stain Index.[23] Each gradable surface was divided into the gingival and body regions. The gingival region was designated as an approximately 2 mm-wide band along the free margin of the gingiva, while the body region was designated as the remaining tooth surface. Extrinsic stain was assessed within each region in terms of both area and intensity, which were quantified using standard four-point scales. In addition to the efficacy and safety assessments, intra-

Table 2. Number of bleeding sites efficacy results[a] – Evaluable subjects.

	Adjusted mean (SE)	Treatment difference (SE)	% Change versus chlorhexidine[b]	P-value; 95% CI of the treatment difference
Week 4				
Two-Step Sequence (n=20)	7.447 (0.779)	2.431 (1.543)	24.6%	0.1246
Chlorhexidine (n=16)	9.878 (1.328)			(-0.708, 5.570)
Week 8				
Two-Step Sequence (n=19)	4.715 (0.697)	3.486 (1.617)	42.5%	0.0385
Chlorhexidine (n=17)	8.201 (1.458)			(0.196, 6.775)
Week 12				
Two-Step Sequence (n=19)	3.840 (0.551)	0.475 (0.884)	11.0%	0.5946
Chlorhexidine (n=16)	4.315 (0.690)			(-1.326, 2.277)

SE = standard error; % = percentage; CI = confidence interval; n = number of subjects.
[a] Via two-sided ANCOVA, where model included baseline and treatment as fixed effect(s), and unequal variances were modeled for each treatment group.
[b] Percent change versus chlorhexidine = 100 × [(chlorhexidine minus two-step sequence)/chlorhexidine].

Table 3. Lobene Composite Stain Index efficacy results[a] – Evaluable subjects.

	Adjusted mean (SE)	Treatment difference (SE)	% Change versus chlorhexidine[b]	P-value
Week 4				
Two-Step Sequence (n=20)	0.103 (0.068)	1.749 (0.429)	94.4%	0.0003
Chlorhexidine (n=16)	1.853 (0.423)			
Week 8				
Two-Step Sequence (n=19)	0.059 (0.043)	2.405 (0.583)	97.6%	0.0002
Chlorhexidine (n=17)	2.463 (0.581)			
Week 12				
Two-Step Sequence (n=19)	0.130 (0.110)	3.148 (0.624)	96.0%	<0.0001
Chlorhexidine (n=16)	3.278 (0.615)			

SE = standard error; % = percentage; n = number of subjects.
[a] Via two-sided ANOVA, where model included treatment as a fixed effect, and unequal variances were modeled for each treatment group.
[b] Percent change versus chlorhexidine = 100 × [(chlorhexidine minus two-step sequence)/chlorhexidine].

oral clinical photographs were collected at baseline and end-of-treatment.

Summary statistics of the demographic characteristics and measurements were calculated for each treatment group and overall. Gingivitis and stain scores were calculated for each treatment group and visit, and comparisons to baseline were investigated using paired-difference t-tests. The treatment groups were compared using the ANCOVA method with baseline as a covariate. In addition, for gingivitis scores, two-sided 95% confidence intervals were calculated. All comparisons were two-sided at the 0.05 level of significance.

Results

Forty-four subjects were eligible and randomized to treatment, 21 in the two-step dentifrice/gel sequence (test) group, and 23 in the chlorhexidine positive-control group. The enrolled study population ranged from 19 to 65 years in age, with a mean age of 36.3 years, and 59% were female. As seen in Table 1, the adjusted mean number of bleeding sites at baseline was 16.6±6.0 in the test group and 17.1±7.2 in the positive-control group; these values did not differ significantly (P= 0.78). Eight subjects, two from the test group and six from the positive-control group, withdrew on or before Week 12. One subject in the positive-control group had non-evaluable data at Week 12, resulting in 35 subjects completing the trial with fully evaluable data: 16 in the control group and 19 in the test group.

Both the test regimen and the positive-control regimen produced similar reductions in the number of bleeding sites during the study versus baseline, with adjusted means of 7.45, 4.72, and 3.84 bleeding sites for the test group, and 9.88, 8.20, and 4.32 bleeding sites for the control group at Weeks 4, 8, and 12, respectively (P≤ 0.0001 for all comparisons vs. baseline). For between-group comparisons of gingival health outcomes, the two-step sequence provided comparable reductions in gingival bleeding sites to the chlorhexidine group at Weeks 4 and 12 (P≥ 0.12), with a significant difference favoring the two-step sequence at Week 8 (P= 0.04; Table 2).

For Lobene composite stain, there were no significant between-group differences at baseline (P= 0.595). Relative to baseline, the chlorhexidine positive-control group had significant increases in composite, area and intensity stain scores (P< 0.003) whereas there were no significant changes in the two-step dentifrice/gel sequence group (P> 0.12). Between-treatment comparisons showed significantly higher composite, area and intensity stain scores for the chlorhexidine group compared to the two-step dentifrice/gel sequence at Weeks 4, 8 and 12 (P≤ 0.0006; Tables 3-5). Differences in stain scores ranged from 92.1% to 97.6%.

Table 4. Lobene Intensity Stain Index efficacy results[a] – Evaluable subjects.

	Adjusted mean (SE)	Treatment difference (SE)	% Change versus chlorhexidine[b]	P-value
Week 4				
Two-Step Sequence (n=20)	0.066 (0.038)	0.763 (0.180)	92.1%	0.0002
Chlorhexidine (n=16)	0.828 (0.176)			
Week 8				
Two-Step Sequence (n=19)	0.037 (0.029)	1.047 (0.243)	96.6%	0.0001
Chlorhexidine (n=17)	1.084 (0.241)			
Week 12				
Two-Step Sequence (n=19)	0.080 (0.067)	1.404 (0.272)	94.6%	<0.0001
Chlorhexidine (n=16)	1.484 (0.264)			

SE = standard error; % = percentage; n = number of subjects.
[a] Via two-sided ANOVA, where model included treatment as a fixed effect, and unequal variances were modeled for each treatment group.
[b] Percent change versus chlorhexidine = 100 × [(chlorhexidine minus two-step sequence)/chlorhexidine].

Table 5. Lobene Area Stain Index efficacy results[a] – Evaluable subjects.

	Adjusted mean (SE)	Treatment difference (SE)	% Change versus chlorhexidine[b]	P-value
Week 4				
Two-Step Sequence (n=20)	0.035 (0.023)	0.631 (0.167)	94.8%	0.0006
Chlorhexidine (n=16)	0.665 (0.166)			
Week 8				
Two-Step Sequence (n=19)	0.026 (0.019)	0.807 (0.192)	96.8%	0.0002
Chlorhexidine (n=17)	0.833 (0.191)			
Week 12				
Two-Step Sequence (n=19)	0.051 (0.041)	1.046 (0.209)	95.3%	<0.0001
Chlorhexidine (n=16)	1.097 (0.205)			

SE = standard error; % = percentage; n = number of subjects.
[a] Via two-sided ANOVA, where model included treatment as a fixed effect, and unequal variances were modeled for each treatment group.
[b] Percent change versus chlorhexidine = 100 × [(chlorhexidine minus two-step sequence)/chlorhexidine].

There were seven adverse events, one in the test group and six in the positive control group. By type, there was one report of tooth sensitivity in the test group, severity was mild, and this occurrence did not impact participation. The six adverse events recorded in the positive-control group included taste alteration (two), tooth sensitivity (two), xerostomia (one) and tooth staining (one), the latter of which contributed to early discontinuation.

Discussion

The antiplaque/antigingivitis effectiveness of chlorhexidine is well demonstrated, making this agent a gold standard for gingival health maintenance.[9-12,16] Yet, this agent requires a prescription, can result in taste alteration,[14,15] and tends to promote tooth staining requiring removal by a dental professional.[12,13,15] These barriers to patient acceptance often limit its use to short-term periods. Stannous fluoride, the active antimicrobial agent in the two-step dentifrice/gel sequence, has demonstrated statistically significant reductions in plaque accumulation and gingival bleeding in numerous published clinical trials.[11,17-22] In this head-to-head investigation, both chlorhexidine and the two-step sequence produced significant reductions in the number of bleeding sites relative to baseline at all time-points (P≤ 0.0001). At Week 12, each treatment had reduced the approximate mean number of bleeding sites from 17 to 4, a significant and meaningful improvement. Importantly, while the study design was planned to assess non-inferiority for the experimental product, measured bleeding site responses were directionally better with the two-step dentifrice/gel sequence compared to chlorhexidine at Week 4 and Week 12, and treatments differed significantly (P= 0.04) at Week 8 favoring the two-step group.

While both groups had significant reductions in gingivitis, esthetic responses differed. Stain accumulation was evident in the chlorhexidine group beginning at the first post-prophylaxis visit (Week 4) and continuing through study completion (Week 12). In contrast, there was no evidence of stain accumulation in the two-step group. As measured, stain accumulation was essentially zero (with the median change in composite stain accumulation of 0.0 after 4, 8 and 12 weeks of test product use), irrespective of the habits and practices of study subjects after prophylaxis. Groups differed significantly (P≤ 0.0006) on stain accumulation at each post-baseline timepoint favoring the two-step group (Figure). Of note, these tooth stain accumulation differences were easily visible with intraoral images as early as Week 4 of this study.

The two-step sequence was well tolerated in this study, producing beneficial effects for gingival health without important adverse safety outcomes. Only one subject in the two-

Figure. Example of stain accumulation with the chlorhexidine positive-control regimen (left) versus the two-step dentifrice/gel sequence regimen (right).

step sequence group reported an adverse event (mild tooth sensitivity), which did not affect participation. Adverse events were more common in the positive control group, and were typical of those previously reported in studies involving chlorhexidine rinses.[12,15] One subject in the positive-control group discontinued the study due to tooth staining.

The gingivitis prevention model used in this study (prophylaxis followed by treatment) was selected due to its applicability to the practice setting, and limited to 3 months post-prophylaxis due to concerns about stain accumulation without intervention in the chlorhexidine group. Results from this 12-week prevention study demonstrated that use of the two-step stannous fluoride dentifrice/hydrogen peroxide whitening gel sequence provided similar or better antigingivitis benefits to chlorhexidine gluconate rinse without attendant stain accumulation. As such, it likely represents a viable, non-prescription alternative for longer term use in practice to manage gingivitis between recall visits.

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Safety and effectiveness of a two-step dentifrice/gel sequence with medication-associated hyposalivation: A randomized controlled trial in a vulnerable population

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ABSTRACT: Purpose: A randomized controlled trial was conducted to evaluate the safety and effectiveness of a two-step dentifrice/gel oral hygiene sequence in a vulnerable population. **Methods:** Prior to the research, institutional review was obtained for the protocol, consent and advertising. The study targeted adults with medication-associated xerostomia, because of the plaque accumulation and possible oral safety risks seen in this population. Eligible subjects with a medication history and measured hyposalivation were randomly assigned to one of two oral hygiene groups: (1) a two-step 0.454% SnF_2 dentifrice and 3% H_2O_2 gel sequence or (2) a regular anticavity toothpaste control. Test products were dispensed with a regular manual brush in blinded over-labeled kits with usage instructions. Subjects were evaluated at baseline and after 2 and 6 weeks of test product use. Safety was assessed as adverse events from clinical examination and interview. Digital plaque image analysis of the anterior facial teeth measured fluorescein-disclosed daytime plaque levels, and unstimulated saliva was collected over a 5-minute period in pre-weighed vials. **Results:** A total of 49 subjects ranging from 31-80 years of age (53% female) were enrolled, and 45 completed Week 6. Only the two-step dentifrice and gel sequence differed significantly (P< 0.005) from baseline on daytime plaque coverage, and salivary flow increased significantly (P= 0.033) in that group as well. Between-group comparisons for daytime plaque favored the two-step sequence with 41-46% improvements in plaque control. At Week 6, adjusted daytime plaque means (SE) were 5.9 (0.7) and 10.0 (1.1) for the two-step and control groups, respectively (P< 0.004). Adverse events were mild in severity, groups differed significantly (P= 0.02) on occurrence, and events did not contribute to dropout. (*Am J Dent* 2018;31:24A-28A).

CLINICAL SIGNIFICANCE: In a randomized controlled trial among a vulnerable population, use of an oral hygiene sequence comprised of stannous fluoride dentifrice and a hydrogen peroxide whitening gel improved daily plaque control without adversely impacting salivary flow or oral health.

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Introduction

Xerostomia is a subjective sensation of oral dryness that is typically associated with salivary gland hypofunction.[1] Contributing factors include autoimmune diseases, surgical, chemical or radiation therapy, infections and others.[2-4] In addition, several hundred common medications, including antihypertensives, antianxiety agents, psychiatric remedies, antihistamines, and others have hyposalivation as a known side effect.[5] The consequences of combinations of xerostomic medications, especially for the population with no or limited insurance, may be severe in the oral cavity.[6] Prevalence is unknown, but a retrospective survey of dental patients suggests that 12% or more may report xerostomia.[7] At-risk groups may present with much higher (60%+) rates of xerostomia.[8] A systematic literature review suggests prevalence may be 27-32% of the medicated population.[8]

For both the general population and specific risk groups, saliva plays a recognized role in oral health. Chronic hyposalivation may contribute to oral diseases and conditions, including caries, sensitivity, tooth surface loss and various oral infections.[5,10] Surveys comparing severe chronic hyposalivation cases like Sjögren's syndrome to controls show significantly higher levels of plaque in the low-to-no salivary flow population.[11,12] In addition to plaque accumulation, research suggests differences in the prevalence and severity of gingivitis and periodontal disease, plus other adverse oral health outcomes. Various interventions have been proposed, though systematic review provides limited evidence of benefits for certain topical and non-drug therapies.[13,14] A recent review emphasizes the role of dentistry in the diagnosis and multidisciplinary management of xerostomia.[10]

Low salivary flow has also been shown to be related to the occurrence of oral mucosal lesions.[15] One study[16] implicated medication use and increased oral mucosal inflammation among US veterans. Behavioral, physiological and other factors may contribute to tissue fragility and healing impairment. Irrespective of the etiology, hyposalivation represents a potentially important model to study both favorable and unfavorable outcomes of interventions. Research involving some case types can be problematic, because of prevalence, access, or overall health risks, as exemplified by radiation-induced xerostomia. Alternatively, medication-associated xerostomia may represent a reasonably "vulnerable" population that is more amenable to clinical research, with broader inference.

Recently, a novel two-step sequence was developed for daily oral hygiene with 0.454% stannous fluoride dentifrice followed by 3% hydrogen peroxide whitening gel. The in-use esthetics with this novel sequence are impressive and unique, and clinical trials with this two-step sequence have shown promising results in a general population without serious oral adverse events.[17] Because toothpaste is generally used, a controlled clinical trial was conducted among individuals with medication-induced xerostomia to ascertain effectiveness and
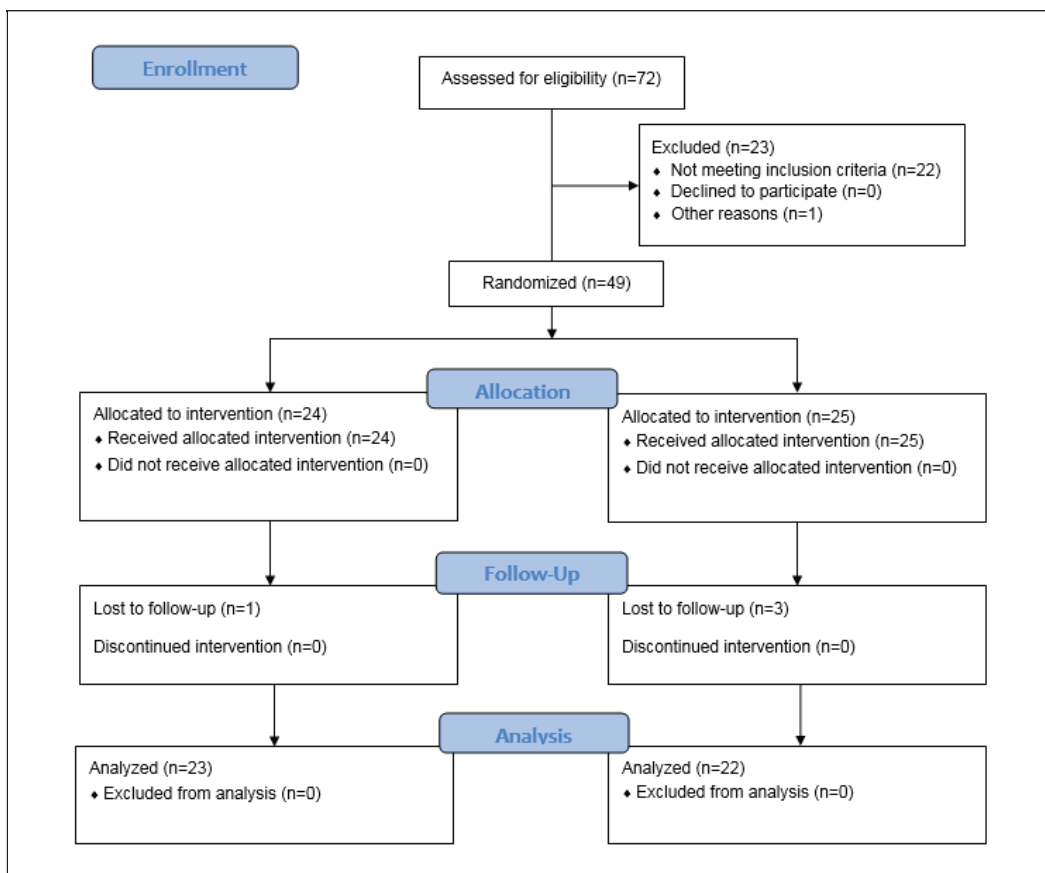
Fig. 1. Subject disposition.

safety of sequential two-step daily oral hygiene in this presumptively vulnerable population.

Materials and Methods

A randomized negatively-controlled clinical trial evaluated the safety and effectiveness of a novel two-step paste/gel oral hygiene sequence using stannous fluoride followed by hydrogen peroxide. The study targeted a vulnerable population, and prior to initiation, the Tufts University Health Sciences Campus Institutional Review Board reviewed (#10576) the study protocol, informed consent and advertising. Subjects with medication-associated xerostomia symptoms were recruited from the Oral Medicine clinic, general School of Dental Medicine, and elsewhere in Boston, Massachusetts, USA. There were four visits: screening, baseline, and after 2 and 6 weeks of treatment. Eligibility was determined at screening, and limited to adult volunteers with overnight plaque accumulation, a xerogenic medication history, and hyposalivation as evidenced by a 5-minute unstimulated salivary flow below 0.2 mL. Subjects were randomly assigned to treatment, and test products were dispensed at baseline for 6 weeks at-home use. Efficacy and clinical safety were measured at baseline, and each post-treatment visit, while salivary flow was measured at screening (for eligibility) and after 6 weeks of treatment.

The clinical trial directly compared two oral hygiene treatment groups: 1) a two-step dentifrice and gel system[a] comprised of 0.454% stannous fluoride dentifrice (step 1) for plaque and gingivitis followed by a 3% hydrogen peroxide whitening gel (step 2). Subjects were instructed to brush two times a day, using step 1 for 1 minute, and then step 2 for the second minute; or 2) 0.76% sodium monofluorophosphate dentifrice[b] (serving as a regular oral hygiene control). Subjects were instructed to brush thoroughly twice daily.

Eligible subjects were randomly assigned to treatment balancing for screening scores. All subjects received a regular manual brush along with marketed instructions as noted above to simulate "real world" usage, and for blinding, all assigned oral hygiene products and printed instructions were dispensed in plain subject-identified kit boxes for at-home unsupervised use.

Efficacy was assessed from daytime plaque levels on the anterior facial dentition, measured instrumentally using a standard image analysis method with daily calibration.[18] Dental plaque was disclosed using 5.0 mL of 1,240 ppm fluorescein dye in a glycerin base rinsed for 1 minute, with before/after rinsing with a phosphate buffer, with all test solutions prepared daily by the Tufts Medical Center pharmacy. Standard orientation and access for illumination were achieved using a chin rest and cheek retractors to allow 45°/0° illumination at a fixed focal distance, consistent with that described for tooth color imaging.[19] A single digital image was collected of the anterior facial dentition using a digital camera and 25 mm lens, polarized ultraviolet flash and portable computer. For each image, quadratic discriminate analysis was used to identify image pixels representing tooth surfaces and disclosed dental plaque surfaces, the latter of which is green under UV illumination. On the 12 anterior teeth, the number of pixels was summed, and plaque area was quantified from pixel counts as percent area coverage (0-100%). Using this instru-

Table. Baseline characteristics.

	Baseline characteristics – All subjects			
	SnF$_2$/H$_2$O$_2$ (N=24)	NaMFP (N=25)	Overall (N=49)	Two-sided P-value
Age (Years)				
Mean (SD)	58.0 (14.3)	56.8 (11.3)	57.4 (12.7)	0.74
Range	39 - 80	31 - 78	31 - 80	
Gender				
Female	13 (54%)	13 (52%)	26 (53%)	0.99
Male	11 (46%)	12 (48%)	23 (47%)	
Unstimulated saliva (mL/5 min)				
Mean (SD)	0.09 (0.07)	0.08 (0.06)	0.09 (0.07)	0.90
Range	0 - 0.20	0 - 0.19	0 - 0.19	
Plaque (Area %)				
Mean (SD)	12.5 (12.8)	11.5 (14.7)	12.0 (13.6)	0.70
Range	1.16 - 57.03	0.01 - 77.32	0.01 - 77.32	

mental approach, all plaque area measurements were collected blind to treatment and period. Baseline and post-baseline (Week 2 and 4) results were compared to quantify change in plaque area coverage over time.

Safety-related measures included assessment of salivary flow and clinical examination to assess possible physiological adverse effects as well as soft tissue irritation. Unstimulated salivary flow was measured after at least 1 hour of daytime fasting. Salivary samples were collected in pre-weighed 50 mL vials every 60 seconds over a 5-minute period. Collected saliva vials were weighed and salivary volume was determined using an assumed density of 1.0 mL/g, after which flow rates were calculated in mL/minute. The oral examination consisted of a thorough evaluation of the oral and perioral region by an experienced dentist who was blinded to treatment assignment. All oral adverse events, irrespective of causality, were recorded for analysis and follow-up.

Demographic data were summarized by treatment and overall. Mean plaque area % responses were compared to baseline using a paired difference t-test, while between-group comparisons used ANCOVA with baseline plaque as a covariate. Salivary flow response was analyzed similarly to plaque. Adverse events were summarized by type and severity using standard pharmaceutical coding practices, and groups were compared on adverse event occurrence and severity using Fishers Exact Test. All comparisons were two-sided using 5% levels of significance.

Results

Informed consent was obtained from 72 adults, 50 met study entrance criteria at screening, 49 had baseline measurements and were randomized and received assigned test products (24 in the two-step group and 25 in the control group). All randomized subjects (Fig. 1) were included in analyses.

The randomized population exhibited considerable diversity. Mean (SD) age was 57.4 (12.73) years, ranging from 31-80, and males and females were similarly represented (Table). All subjects (100%) presented with at least one medication where hyposalivation is a recognized side effect. Of these, antihypertensive and antianxiety medications were most common. In addition, all subjects exhibited hyposalivation at screening, with the overall mean 0.085 mL unstimulated saliva collected in a 5-minute period.

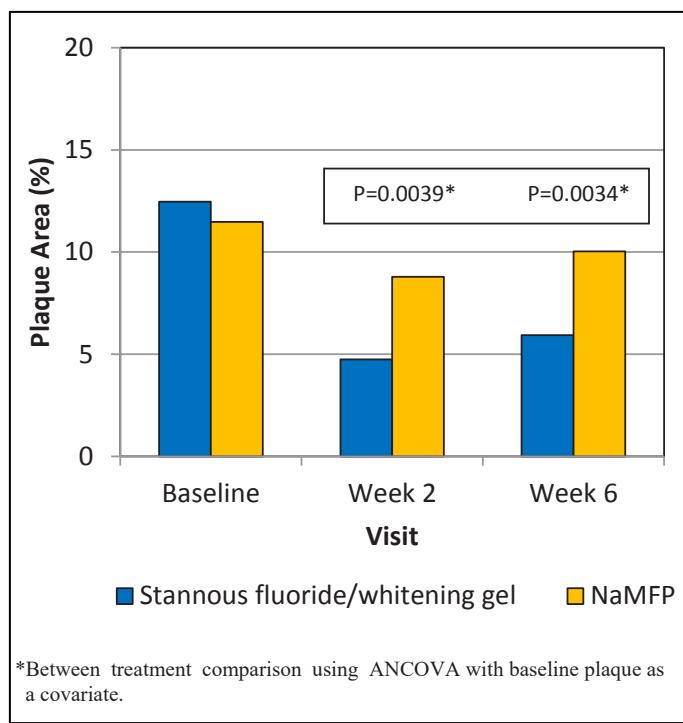*Between treatment comparison using ANCOVA with baseline plaque as a covariate.

Fig. 2. Daytime plaque (Area % Coverage) by group.

At baseline, individual daytime plaque varied from negligible levels to more than three-quarters of tooth surfaces covered. The overall mean (SD) area was 12.0% (13.6), and groups were balanced (P> 0.70) on daytime plaque. Relative to baseline, only the two-step stannous fluoride dentifrice/ hydrogen peroxide gel hygiene sequence yielded significant (P< 0.005) daytime plaque control. Plaque reduction effects were evident at the first treatment visit (Week 2) and persisted through the last treatment visit (Week 6). Between-group comparisons showed significant (P< 0.004) improvements in plaque control ranging from 41-46% for the two-step group relative to the control (Fig. 2).

Safety assessments included salivary flow measurements and adverse events. For saliva, the Week 6 mean (SD) 5-minute unstimulated salivary sample means were 0.15 (0.127) and 0.13 (0.09) in the two-step sequence and control groups, respectively. Only the two-step stannous fluoride plus hydrogen
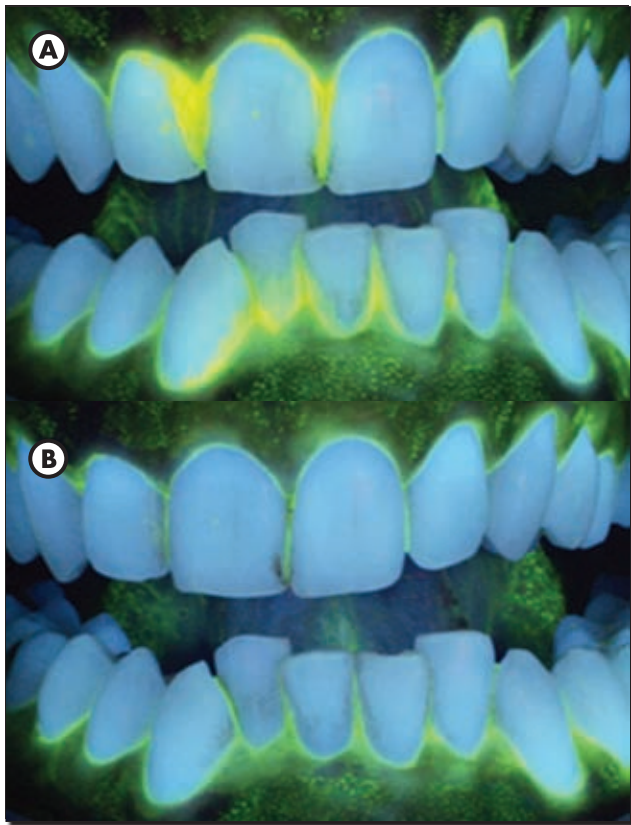
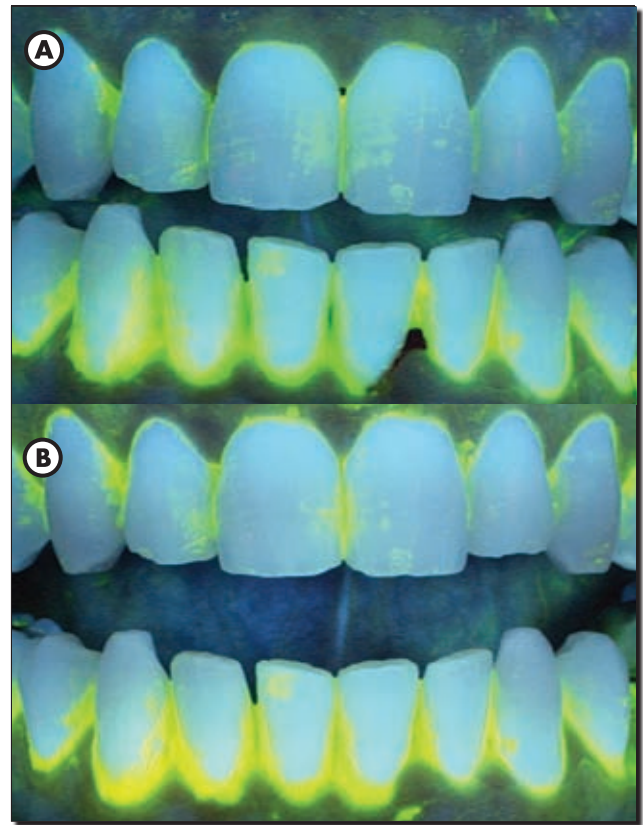Fig. 3 A, B. Daytime plaque at Baseline and Week 2 (Two-Step Group).

Fig. 4 A, B. Daytime plaque at Baseline and Week 2 (Control Group).

peroxide hygiene sequence demonstrated a significant (P< 0.04) increase in salivary flow versus baseline levels. Between-group comparisons were not significantly different. There were eight adverse events reported or observed during the study, involving both treatment groups. Four were observed on clinical examination, and by group, included two examples of minor desquamation and one example of tongue irritation in the two-step sequence, and one example of palatal irritation in the control group. Groups differed (P< 0.02) on adverse event occurrence, all of which was mild in severity, and none of the occurrences contributed to dropout.

Discussion

This study compared plaque response of an oral hygiene sequence comprised of a stannous fluoride dentifrice and a hydrogen peroxide whitening gel versus a regular dentifrice. Assigned test products were dispensed blind to treatment, and used at-home following the specific marketed instructions for each product, with outcomes measured instrumentally without bias. Results demonstrated significant plaque reductions relative to the control group beginning at Week 2, and persisting through Week 6. In this study of daytime plaque accumulation, use of a 0.454% stannous fluoride dentifrice/3% hydrogen peroxide gel sequence reduced daytime plaque accumulation by 40-50% versus regular oral hygiene.

The measured plaque effects were both generalized and visually evident. Most (92%) of the subjects who used the novel sequence had lower measured plaque levels at Weeks 2 and 6, ranging up to 99% reductions versus baseline levels. These outcomes were observed irrespective of starting levels; even those individuals with high baseline plaque exhibited

appreciable reductions over time. Such improvements were readily apparent in the digital images used to measure plaque area, even among subjects with appreciable tooth malalignment that may affect brushing (Figs. 3 A,B). Response differed for the control group, which overall, failed to exhibit either significant measured plaque effects or appreciable visual improvement over time (Figs. 4 A,B).

Of note, the plaque effectiveness was observed without important adverse safety outcomes, and that may be particularly noteworthy given the population and test products in the clinical trial. With respect to the population, the study targeted adults with medication-associated hyposalivation. This population represents a potentially vulnerable population for oral safety, because of the possible impact of hyposalivation on oral mucosa responses.[15] Research suggests that unstimulated whole salivary flow rates of 0.12 - 0.16 mL/minute as the critical range separating individuals with salivary gland hypofunction from those with normal gland function.[20] With respect to the test products, one treatment group received a two-step sequence that included instructions specifying 1-minute brushing with a 3% hydrogen peroxide gel (the second step in this assigned hygiene regimen). Salivary peroxidase has long been identified as having a presumptive role in peroxide decomposition.[21] Differences in oral irritation were observed, but these were minor, and importantly, did not contribute to dropout. Clinical safety of topical peroxide application has previously been studied among individuals with medication-induced xerostomia within the context of esthetic tooth whitening.[22] The new research extends the merits of xerostomia as a model population for safety assessment to other forms of topical peroxide delivery, including dentifrices.

In addition to the safety findings, the research on xerostomia yielded an unexpected outcome. There was a significant (P< 0.05) increase in daytime unstimulated salivary flow in the two-step oral hygiene group. While the amount was relatively small (+0.06 mL/5 minute), this represented approximately a 72% increase above the baseline level. The xerostomia in the study was medication-associated, and in contrast to Sjögren's syndrome or similar conditions, it may be reversible with stimulation.[1] The mechanism remains unknown, and of course, further research is needed to ascertain whether this favorable effect on salivation is real and reproducible, and whether it contributes to other positive health or experiential outcomes. What is clear, however, is that use of stannous fluoride followed by hydrogen peroxide did not limit salivary flow relative to baseline or control in this vulnerable population study.

Overall, this research showed a significant and consistent reduction in daytime plaque following use of a stannous fluoride plus hydrogen peroxide oral hygiene sequence. The study was conducted among individuals with hyposalivation, as this population may have an added risk for oral irritation.[23] Safety outcomes in the new study were consistent with other general clinical research using this novel sequential oral hygiene technology.[17] To date, clinical testing in vulnerable populations remains uncommon, but research such as this may provide important evidence on the overall safety and tolerability with broader use.

a. Marketed as Crest Pro-Health [HD] and Oral-B [HD] depending on the region; The Procter & Gamble Company, Cincinnati, Ohio, USA.
b. Colgate Cavity Protection, Colgate-Palmolive, New York, New York, USA.

Dr. Singh is Associate Professor, Department of Diagnostic Sciences; Dr. Papas is Johansen Professor of Dental Research and Head of the Division of Oral Medicine, Department of Diagnostic Sciences. Dr. Gerlach is a Research Fellow in Global Oral Care, The Procter & Gamble Company, Mason, Ohio, USA and Adjunct Professor at Tufts University School of Dental Medicine, Boston, Massachusetts, USA,

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