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

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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 anticavity 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≥ 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-

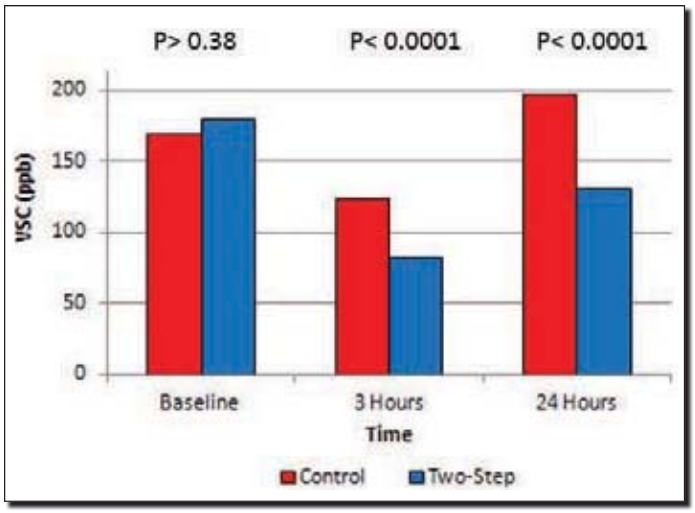

Fig. 1. Treatment comparisons for VSC by visit and group.

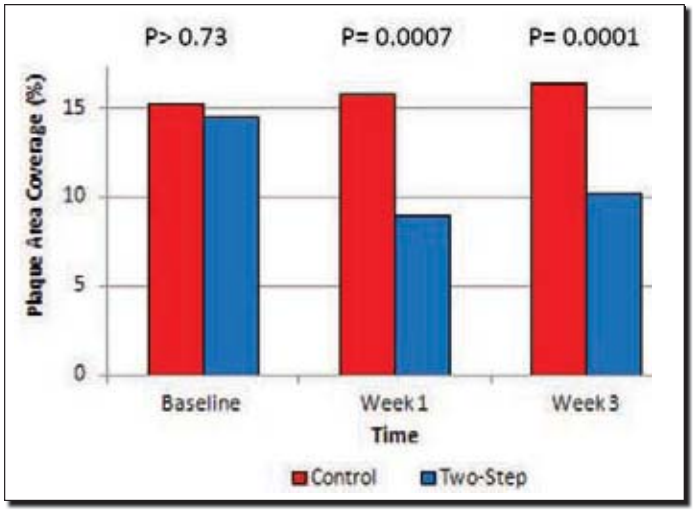

Fig. 2. Treatment comparisons for Plaque Area (%) by visit and group.

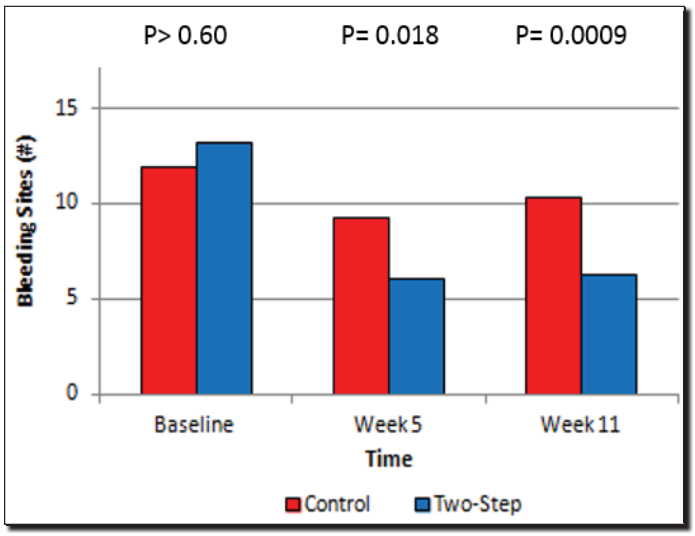

Fig. 3. Treatment comparisons for Gingival Bleeding Sites (#) by visit and group.

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

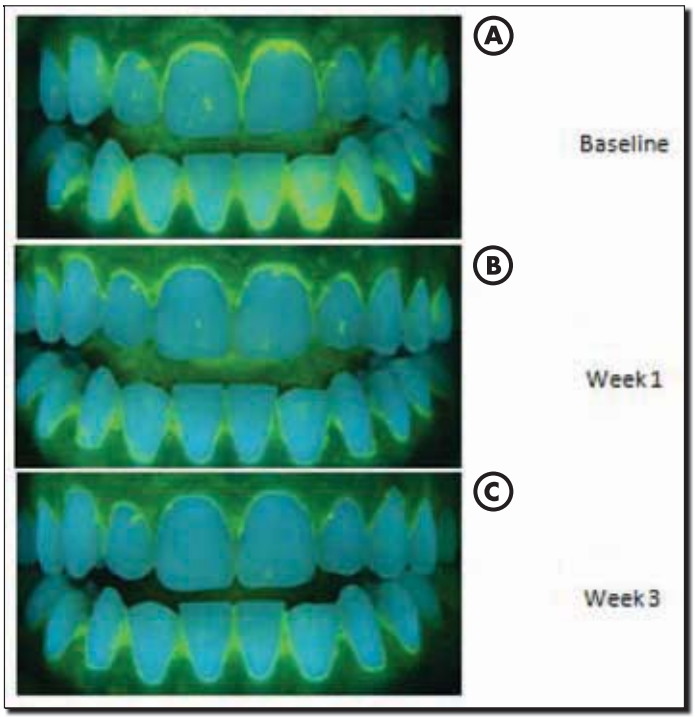

Fig. 4A-C. Overnight plaque at Baseline, and after 1 and 3 weeks use of oral hygiene sequence.

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 appreciable 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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