It is well known that good plaque control is essential for optimizing periodontal health, and that this can be achieved providing an effective daily oral hygiene regimen is adopted. The importance of having a twice-daily, mechanical brushing routine as the basis for ensuring good oral health is well understood, but actually achieving effective plaque removal can still be a challenge for many individuals. Manufacturers respond to the need for optimal plaque control by designing products with features specifically aimed at improving effectiveness. Comparative clinical studies play an important role in differentiating between products, and the results assist professionals in making choices between products based on this evidence. Comparative clinical studies play an important role in differentiating between products, and the results assist professionals in making choices between products based on this evidence.

Comparative clinical studies of novel toothbrushes that focus on assessing the mechanical aspects of plaque removal effectiveness are common. However, an important aspect of plaque control is the prevention of plaque build-up, and in this respect the choice of dentifrice can be critical. Dentifrices with an active ingredient that has the ability to inhibit the formation of new plaque have clear advantages for the user. Stannous fluoride is an agent with known antimicrobial and plaque prevention properties. In 2005, a stabilized stannous fluoride dentifrice was introduced with the cosmetic agent sodium hexametaphosphate. This formulation provides the benefits of stannous fluoride (protection against caries, plaque, gingivitis, erosion, dentinal hypersensitivity, and oral malodor) along with anti-calculus and extrinsic stain removal properties from sodium hexametaphosphate. Further developments have been made to this formulation chassis, and in a novel dentifrice formulation the bioavailability of stannous fluoride has been further stabilized by including stannous chloride (as a sacrificial stannous salt). The bioavailability of stannous fluoride has been cited by other authors as a key aspect of delivering clinically efficacious products. The relative plaque inhibition effects of these novel dentifrices were evaluated in the present study by comparing it with a long-established, marketed dentifrice in which stannous fluoride is combined with amine fluoride (AmF/SnF2) for which an inhibitory action on the formation of new plaque is promoted. Comparing the novel SnF2 formulation to an established product which also contains SnF2 is of interest to dental professionals as they assess relative product efficacy.

It is now possible to measure plaque levels in clinical studies using an objective and sensitive methodology, namely digital plaque image analysis (DPIA). This system has revealed product differences in plaque coverage in randomized controlled clinical studies, and was used to compare plaque inhibitory effects in this comparative clinical study.

Randomized Digital Plaque Imaging Trial Evaluating Plaque Inhibition Efficacy of a Novel Stabilized Stannous Fluoride Dentifrice Compared with an Amine Fluoride/Stannous Fluoride Dentifrice

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Abstract

• **Objective:** To assess the plaque inhibition efficacy of a novel 0.454% stabilized stannous fluoride test dentifrice (SnF2) to an amine fluoride/stannous fluoride marketed control dentifrice (AmF/SnF2) using digital plaque imaging analysis (DPIA).

• **Methods:** The 10-week study was a randomized, two-treatment, three-period, double-blind crossover design. Subjects brushed twice daily with their assigned dentifrice (SnF2 or AmF/SnF2) using a standard manual toothbrush during three treatment periods each lasting 17 days, separated by four-day washout periods. DPIA was used to analyze plaque coverage on facial surfaces of the 12 anterior teeth (canine to canine) by three assessments on Days 15, 16, and 17 at the end of each treatment period. Assessments were conducted after 40 seconds of full mouth brushing with the assigned dentifrice (A.M. post-brush), and during the afternoon (P.M.).

• **Results:** Twenty-seven subjects were randomized and completed the study. At each assessment time point, plaque levels for the SnF2 dentifrice were statistically significantly lower compared to those for the AmF/SnF2 dentifrice (21.4%, 22.6%, 24.3%, respectively; p < 0.0001 for all).

• **Conclusion:** Plaque coverage, as assessed by DPIA, was significantly lower with a novel SnF2 dentifrice than with the AmF/SnF2 dentifrice. The plaque control benefits of the SnF2 dentifrice seen at the morning and afternoon time points indicated significantly better inhibition of plaque re-growth.

(J Clin Dent 2012;23:71–75)
Materials and Methods

Treatment Products

The dentifrice tested in this study had the following formulation: 1450 ppm total F dentifrice (0.454% stabilized SnF₂ and 0.078% sodium fluoride) with stannous chloride and sodium hexametaphosphate (SHMP), referred to here as “SnF₂ test dentifrice.” This dentifrice is marketed as Blend-a-med® Pro-Expert Clinic Line, Gum Protection (Procter & Gamble, Gross Gerau, Germany). The comparison dentifrice was an amine fluoride/stannous fluoride (1400 ppm total F, with 1050 ppm F from AmF and 350 ppm F from SnF₂) dentifrice marketed as Meridol® (GABA International AG, Basel, Switzerland) and referred to here as “AmF/SnF₂.”

Subjects, Study Design, and Procedures

The study subjects were 27 employees at Procter & Gamble (Egham, UK) who gave their informed signed consent to participate. The Institutional Ethics Review Committee had previously approved the application of DPIA methodology for dentifrice research, and all subjects had previously participated in plaque trials that had used this methodology. The study was conducted over a period of 10 weeks and had a double-blind, two-treatment, three-period, randomized crossover design similar to that described by Bellamy, et al.19 (Figure 1). There was an acclimatization period of at least five days, followed by three 17-day treatment periods, each separated from each other by four-day washout periods. Plaque was assessed before and after brushing (A.M. pre-brush and A.M. post-brush) and in the afternoon (P.M.) on Days 15, 16, and 17 using the DPIA method.

Recruitment was carried out by the study administrator among the on-site population at the Egham campus. A brief description of the study and participant requirements were supplied, enabling potential subjects to volunteer. At this point they were provided with full informed consent and verbal explanation. After giving their consent to participate, subjects received an oral soft tissue examination and were assessed for study eligibility according to the study inclusion/exclusion criteria. The main inclusion criteria were: being in good general health; agreeing to the use of treatment products as directed; refraining from eating and drinking (except water) on the morning of the DPIA assessments or within 30 minutes of an afternoon DPIA assessment; and agreeing not to use oral hygiene products, such as electric toothbrushes, chewing gum, or dental floss (although subjects who used floss could continue to floss their back teeth only as these teeth were not imaged, if they were consistent throughout the study, and subjects with a regular gum chewing habit could continue throughout if their routine was regular). The main exclusion criteria were as follows: poor dental health (e.g., rampant caries, severe gingivitis, advanced periodontitis); the use of antibiotics, antimicrobial mouthwash, medicated lozenges, or chlorhexidine two weeks prior to the study or during the study or washout period; dental conditions (e.g., orthodontic appliances) that would interfere with the study; color-matched restorations present on the facial surfaces of the 12 anterior teeth (canine to canine: six mandibular and six maxillary); known dye allergies; pregnancy or breastfeeding. Subjects needed to demonstrate at least moderate plaque formation overnight and during the day. Subjects not forming at least 4.5% plaque coverage as measured by DPIA after overnight accumulation were excluded. During this screening period, subjects brushed ad lib twice per day for at least five days with a standard fluoride toothpaste (Crest® Decay Prevention 0.321% sodium fluoride; Procter & Gamble, Gross Gerau, Germany) and a manual toothbrush (Oral-B® P35 Indicator, medium hardness; Procter & Gamble, Newbridge, Ireland).

For at least five days prior to the start of the first treatment period (i.e., during the acclimatization period) and during the four-day periods that separated the 17-day treatment periods (i.e., the washout periods), subjects were instructed to brush their teeth as normal twice a day (i.e., morning and just before going to bed in the evening) without the use of other oral hygiene products or aids. They were directed to use a wide ribbon of standard NaF dentifrice (Crest Decay Prevention 0.321% sodium fluoride) on a manual toothbrush (Oral-B® P35 Indicator, medium hardness).

Treatment Period 1 started on a Monday, and subjects were randomized to one of four treatment sequence groups (ABB, BAA, ABA, or BAB) where A and B represent the two treatment dentifrice products; test dentifrice (SnF₂) or comparison dentifrice (AmF/SnF₂) which were both supplied in white unbranded packaging to ensure blinding. A randomization allocation table was automatically generated by software designed for the purpose and approved by the statistician in advance of the first
period with each subject’s individual code randomly assigned to a sequence in the table. Subjects were instructed to brush as normal twice a day using a wide ribbon of the supplied toothpaste along with the Oral-B P35 manual toothbrush provided. Subjects were required to brush the inner (lingual) surfaces of their teeth, for brushings on Monday, Tuesday, and Wednesday evenings of each week. There were no instructions on brushing length (i.e., ad lib). The reason for lingual-only brushing was to allow for any effects of the dentifrice to be experienced without disturbing plaque growth over a 24-hour period on the outer facial surfaces that were to be assessed for plaque levels. Subjects were also instructed that evening brushing should be no later than 11:00 p.m. and that there should be no food or drink (except water) afterwards. Plaque was evaluated by DPIA on Days 15, 16, and 17 (Tuesday to Thursday). This gave the subjects time to acclimate to their treatment products. Subjects were instructed not to brush, eat, or drink (except water) on assessment mornings, and were required to attend clinic imaging appointments on these mornings without brushing their teeth beforehand. Subjects disclosed their pre-brushing plaque by first rinsing the mouth for 10 seconds with 25 ml of phosphate buffer (pH 6.0), then rinsing the mouth for one minute with 5 ml of disclosing dye (fluorescein diacetate solution, made fresh every day), followed by rinsing the mouth three times for 10 seconds each with 25 ml of phosphate buffer. Then, between 30 seconds and one minute after the final buffer rinse, DPIA was conducted to assess the A.M. pre-brushing disclosed plaque on the 12 anterior facial tooth surfaces. Using their assigned treatment dentifrice (A or B) and an Oral-B P35 manual toothbrush, subjects then brushed their full mouth for 40 seconds, self-timed. Subjects then re-disclosed their plaque with dye solution and this was followed by DPIA to assess the A.M. post-brushing disclosed plaque. Subjects were then free to conduct their usual activities until their return to the clinic in the afternoon (between 1:30 and 3:30 p.m.) when they disclosed their plaque as before, and had a further (P.M.) DPIA assessment. So as not to interfere with the DPIA assessment process, subjects were instructed that no food or drink was to be consumed during the 30 minutes immediately before the afternoon assessment.

A four-day washout phase, during which subjects returned to using the standard NaF dentifrice and Oral-B P35 manual toothbrush, separated the evening of Day 17 of Period 1 and the start of the second 17-day period (Period 2) the following Monday, and likewise between Periods 2 and 3. The methods for Periods 2 and 3 were the same as those for Period 1, except that the subjects used the assigned treatment product (A or B) according to their assigned treatment sequence. At the end of the study, subjects had a final soft tissue examination.

**Image Analysis**

DPIA, as described previously by Bellamy, et al.,17-19 was used to capture and analyze visible plaque coverage. The region of interest was pre-defined as the facial surfaces of the anterior 12 clearly visible teeth (i.e., six mandibular and six maxillary; canine to canine), and using computer analysis every pixel in the region of interest was assigned to one of four separate classes: tooth; gums; plaque; or background. The following equation was used to calculate the percentage of the visible tooth area covered with plaque:

\[
\text{% plaque coverage} = \left( \frac{\text{plaque pixels} \times 100}{\text{tooth pixels} \times 100} \right)
\]

An expert in image analysis, blinded to the assigned treatment, checked the computer analysis results for consistency and accuracy so images not well classified could be excluded, although this was not necessary in the present study.

**Statistical Methods**

In order to achieve a statistical power of at least 85%, 24 subjects needed to complete this trial to detect a significant mean difference between treatments for the plaque area coverage using a two-sided 5% significance level. This power estimate assumed that the mean treatment difference divided by the residual standard deviation (effect size) was approximately 0.802 or higher in this three-period, crossover design. The percentage plaque area coverage measurements from each of three days were averaged separately for each subject, period, and timepoint (A.M. pre-brush, A.M. post-brush, and P.M.). The outcome of primary interest involved plaque regrowth, primarily overnight plaque and then daytime plaque. For each time point, analysis of variance for the crossover design (general linear mixed model) was used to compare the percent plaque area coverage between treatments, using period and treatment dentifrice as fixed effects and subject as a random effect. The carryover effect was tested for each timepoint, was not statistically significant (p > 0.50), and was subsequently removed from each statistical model. All statistical comparisons were two-sided using a 0.05 significance level.

**Results**

A total of 27 subjects were enrolled and randomized to treatment. All subjects completed all measurements, with the exception of seven subjects who each missed all three timepoints from one study period (two subjects from period 1, two subjects from period 2, and three subjects from period 3). No subject experienced any adverse effects of treatment during the study. The ages of the subjects ranged from 20 to 56 years (mean of 34.2 years and standard deviation of 8.32); 48% of the subjects were female (Table I).

<table>
<thead>
<tr>
<th>Subjects: n (%)</th>
<th>Age: Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>14 (51.9)</td>
<td>13 (48.1)</td>
</tr>
</tbody>
</table>

SD = standard deviation.

During the 17-day usage period, subjects demonstrated significantly lower plaque area coverage while using the SnF₂ test dentifrice than while using the AmF/SnF₂ marketed dentifrice. This was true for all three timepoints; A.M. pre-brushing, A.M. post-brushing, and P.M. There was no statistically significant (p > 0.09) period effect at any time point. Table II shows the mean plaque coverage for each timepoint and the statistical treatment comparisons between the products.
of the SnF$_2$ on the bacterial metabolism, which could result in
in the AmF/SnF$_2$ dentifrice group at six months, plaque levels
reductions in pathogenicity without changing plaque accumula-

The SnF$_2$ test dentifrice offered significant plaque inhibition
in combination with a NaF rinse. In a population exhibiting
poor oral hygiene and high gingivitis scores, all subjects
improved dramatically in oral hygiene over the nine-month study,
with no observable difference between the treatment groups in
terms of plaque scores.

This brief review suggests the efficacy of AmF/SnF$_2$ combined
in a dentifrice is mixed, with some favorable results suggesting
clinical efficacy, while other research is inconclusive. However,
the long established, marketed comparator dentifrice tested in this
study is seen by many dental professionals as the benchmark
toothpaste for gingivitis efficacy. Therefore, comparing this
established marketed product to a novel formulation also con-
taining SnF$_2$ is of significant interest to dental professionals
looking for clinically proven and effective products to recom-
med to patients with gingival health problems.

The plaque inhibition benefit of stabilized SnF$_2$ in dentifrice
formulations containing the cosmetic agent sodium hexameta-
phosphate (SHMP) has been well established. Additional ben-
fits have also been reported in the areas of gingivitis, hyper-
sensitivity, oral malodor, calculus, and extrinsic staining.
For a population identified as having gingival health problems,
increasing bioavailability of the active stannous fluoride could
potentially improve the clinical outcomes. Therefore, the original
SnF$_2$ formulation with SHMP has been modified to stabilize the
bioavailability of stannous fluoride via the addition of stannous
chloride as a source of “sacrificial” stannous salt.

This novel SnF$_2$ test dentifrice has been previously tested for
plaque inhibition performance in comparison to an aluminium
fluoride/chlorhexidine digluconate dentifrice (AlF$_3$/Chx). Chlor-
hexidine is known to be an effective agent for plaque in-
hibition when formulated into a mouthrinse, but the effects may
be less pronounced in a dentifrice due to potential interactions of
chlorhexidine with other dentifrice excipients. The results
showed that the SnF$_2$ dentifrice provided superior (p < 0.05)
plaque inhibition compared to the AlF$_3$/Chx dentifrice, both
during the day and overnight, in a randomized, blinded, crossover
study using DPIA.

In the study reported here, the novel SnF$_2$ test dentifrice was
again significantly better at inhibiting plaque compared to the
marketed comparison dentifrice, which this time contained
AmF/SnF$_2$ in combination. Relative percent reductions in mean
plaque coverage for the SnF$_2$ dentifrice versus the marketed
AmF/SnF$_2$ dentifrice were 21.4% and 24.3% at the A.M.
pre-brushing and afternoon time points, respectively, and
immediately after brushing, the SnF$_2$ test dentifrice demonstrated significantly
(p < 0.0001) less plaque coverage by 22.6% relative to the
 marketed comparison dentifrice, which this time contained
SnF$_2$ is of significant interest to dental professionals
looking for clinically proven and effective products to recom-
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both treatment comparisons were highly statistically significant
(p < 0.0001). Additionally, the SnF$_2$ test dentifrice demonstrated significantly
(p < 0.0001) less A.M. post-brushing plaque on
average with a relative reduction of 22.6%, indicating that the
overnight plaque inhibitory advantage of this dentifrice was con-
tinued during plaque removal.

The SnF$_2$ test dentifrice offered significant plaque inhibition
advantages relative to the AmF/SnF$_2$ dentifrice in the present 10-
week evaluation. When formulated with a proven anti-tartar/
anti-stain agent (sodium hexametaphosphate) to mitigate the
potential side effect of mild extrinsic staining associated with
some stannous fluoride products, the result is an effective, and

### Table II

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Dentifrice Treatment</th>
<th>Estimated Mean (SE)</th>
<th>Relative % Reduction</th>
<th>Comparison p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.M. Pre-brush</td>
<td>SnF$_2$</td>
<td>14.36 (1.27)</td>
<td>21.38</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>AmF/ SnF$_2$</td>
<td>18.27 (1.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.M. Post-brush</td>
<td>SnF$_2$</td>
<td>6.25 (0.81)</td>
<td>22.58</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>AmF/ SnF$_2$</td>
<td>8.07 (0.81)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.M.</td>
<td>SnF$_2$</td>
<td>10.75 (1.07)</td>
<td>24.29</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>AmF/ SnF$_2$</td>
<td>14.20 (1.07)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE = standard error.

Two-sided p-value; analysis of variance for crossover design.

Overnight plaque growth (i.e., A.M. pre-brushing) when subjects were using the SnF$_2$ test dentifrice was significantly
(p < 0.0001) lower on average by 21.4% than when subjects used the AmF/SnF$_2$ marketed dentifrice, with estimated means (SE)
of 14.36 (1.27) and 18.27 (1.27), respectively. Immediately after
brushing, the SnF$_2$ test dentifrice demonstrated significantly
(p < 0.0001) less plaque coverage by 22.6% relative to the
AmF/SnF$_2$ marketed dentifrice, with estimated means (SE)
of 6.25 (0.81) and 8.07 (0.81), respectively. After a period of day-
time plaque re-growth, the SnF$_2$ test dentifrice had significantly
(p < 0.0001) less plaque coverage on average by 24.3% versus the
AmF/SnF$_2$ marketed dentifrice, with estimated means (SE)
of 10.75 (1.07) and 14.20 (1.07), respectively.

### Discussion

Amine fluoride and stannous fluoride have been used in com-
bination in dentifrice products (primarily in Europe) for many
years, with Meridol (principally marketed in Germany) being the
main example. Research testing the efficacy of this active com-
bination has been published. Bánóczy, et al. tested AmF/SnF$_2$
in various forms; in a dentifrice formulation, in a mouthrinse
formulation, and in a regimen using both. A non-fluoridated
placebo control dentifrice was also used in the 12-week, parallel-
group, double-blind study. Plaque was evaluated at the start and
end of the treatment period. The mean scores of dental plaque
decreased in all four groups, with the reduction being significant
(p < 0.05) in all groups except the placebo toothpaste group. Clinically
significant reductions in gingivitis were seen in the same
treatment groups, with the authors concluding the clinical efficacy of
the AmF/SnF$_2$ combination in both dentifrice and rinse forms had
been proven. Sgan-Cohen, et al. tested an AmF/SnF$_2$
in a six-month trial compared to a NaF dentifrice control. Plaque,
gingivitis, and bleeding on probing were all evaluated. Although
gingivitis and bleeding scores were significantly lower in the
AmF/SnF$_2$ dentifrice group at six months, plaque levels
were not significantly lower at any measured time point (three
weeks, three months, or six months) during this trial. The authors
concluded this was potentially due to the mechanism of action
of the SnF$_2$ on the bacterial metabolism, which could result in
reductions in pathogenicity without changing plaque accumulation.
Mengel, et al. compared an AmF/SnF$_2$ dentifrice to a NaF
toothpaste (negative control), where both dentifrices were used
in combination with a NaF rinse. In a population exhibiting
poor oral hygiene and high gingivitis scores, all subjects
improved dramatically in oral hygiene over the nine-month study,
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This novel SnF$_2$ test dentifrice has been previously tested for
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some stannous fluoride products, the result is an effective, and

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consumer-acceptable product for patients needing improved plaque control. One limitation of the trial was that it did not include a negative control dentifrice, so we are not able to comment on the plaque inhibition efficacy of the AmF/SnF$_2$ dentifrice beyond that of a standard fluoride dentifrice.

The management of dental plaque and the prevention of periodontal disease can be achieved through the proper use of dental products, most commonly dentifrices and toothbrushes. But the worldwide prevalence of caries and gingivitis and other oral health conditions is evidence of the need for improved products and greater awareness in individuals. $^{24-26}$ Randomized controlled clinical trials serve to differentiate between products and provide the efficacy data needed for making informed choices. Many of the recent advances in dentifrice formulations focus on offering multiple benefits within a single product, and are clearly a convenient means of achieving a full range of oral health benefits. It would appear that by maximizing the stannous in its formulation and by the use of stabilized stannous fluoride, the test dentifrice in this study provides individuals with a product capable of giving highly effective plaque control.

Conclusions

Plaque levels during 17 days of treatment with a novel stabilized 0.454% stannous fluoride (with stannous chloride and sodium hexametaphosphate) test dentifrice were significantly ($p < 0.0001$) lower than with a marketed comparator amine fluoride/stannous fluoride dentifrice when measured by DPIA at morning (pre-brushing) and afternoon time points, indicating better inhibition of overnight and daytime plaque accumulation, as well as after-morning brushing.

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