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Effectiveness of a novel amine + zinc + fluoride toothpaste in reducing plaque and gingivitis: results of a six-month randomized controlled trial

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Abstract

Objectives To evaluate the efficacy of a toothpaste containing amine compound, 0.5% zinc lactate, and 1400 ppm F (NaF) in comparison to a regular fluoride toothpaste containing 1450 ppm F as MFP/NaF (negative control). Plaque and gingivitis indices were determined over a 6-month period.

Materials and methods In a randomized, double-blind, two-cell, parallel-group design on healthy adults from the Bangkok, Thailand area presenting with initial gingivitis (Löe-Silness gingival index ≥ 1) and visible plaque (Turesky Modification-Quigley-Hein index ≥ 1.5) were assigned test and negative control toothpastes and instructed to brush twice daily for two minutes.

Results Site-level ($N = 10,778$) and subject-level ($N = 92$) data indicated that the test toothpaste increased the number of healthy sites and improved gum health as compared to the negative control toothpaste. For the test group, after 6 months, the average reduction for plaque, gingival index, and gingival severity was 31.2%, 32.3%, and 49.3%, respectively. Average reductions were greater in the test group than the negative control group across all measures.

Conclusions The amine + zinc + fluoride toothpaste significantly improved measures of plaque (12× more effective) and gingivitis (5× more effective) in comparison to a standard fluoride toothpaste, suggesting that the novel formulation with antibacterial active ingredients amine and zinc facilitates more effective plaque removal, and promotes gingival health.

Clinical relevance These results suggest that daily use of amine + zinc + fluoride toothpaste can provide a significant improvement in managing plaque accumulation and bleeding associated with gingival inflammation especially for patients experiencing gingivitis.

Clinical trial registration NCT06563518 (registration date: August 19, 2024).

Keywords Amine, Fluoride, Gingivitis, Dental plaque, Toothpaste

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Introduction

Approximately half of children and adults in the United Kingdom have gingivitis [1, 2]. In the U.S., estimates vary, but prevalence is as high as 55% of adults for some groups [3]. Prevalence rate estimates of gingivitis and the clinical definitions have recently changed. A uniform definition of gingivitis was developed in a joint European Federation of Periodontology (EFP) and American Academy of Periodontology (AAP) workshop in 2017 [4]. Previous gingivitis data collection was based on differing definitions of gingival health, which explains the large discrepancies in estimates to date. However, a recent study of the global burden of periodontal disease indicates that prevalence and incidence are worsening and highly variable by world region [5].

It is well recognized that gingivitis disease severity is highly positively associated with age. As demographics shift and the population ages, the patient- and system-level healthcare burden of gingivitis and periodontal disease continues to rise [6, 7]. Gingivitis itself can be reversed; however, if gingivitis goes untreated, it can lead to periodontitis, a more advanced stage of periodontal disease [8, 9]. Untreated or insufficiently treated advanced periodontal disease is associated with severe destruction of the tissues supporting teeth and eventually leads to tooth loss [10]. Non-surgical periodontal therapy is a gold standard for treatment; however, dentifrices may be considered earlier in the treatment process as a less invasive, accessible, and cost-effective intervention, especially in mild cases or as a complement to professional therapies. Healthcare costs associated with periodontal disease in Europe exceed €150 billion [11]. Periodontal disease contributes to lost productivity and disability [12]. Managing gingivitis is a key prevention strategy to prevent more advanced stages of periodontal disease, preventing the significant burden periodontal disease places on individuals and healthcare systems.

When impacted by gingivitis, the gingival tissue becomes swollen, red, and tender [4]. Clinically, the inflammation is characterized by spontaneous bleeding and/or bleeding that occurs when touched or probed [4]. Teeth with consistently inflamed sites are less likely to remain intact over the course of patients' lives [13]. Over time, gingivitis assessments have become increasingly empiric and less reliant on subjective clinical assessments of redness and swelling [9]. Gingivitis is now measured as the site-level and patient-level [4, 9]. Sites are evaluated visually and are assessed for bleeding on probing (BOP); the percentage of sites with BOP indicates whether, at the patient level, there is a diagnosis of a gingivitis case [8]. A gingivitis case is diagnosed if the BOP score is equal to or greater than 10% with the absence of detectable attachment loss due to periodontitis [9, 10].

Gingivitis is most commonly caused by plaque [14]. Gingivitis measures can be improved by managing plaque biofilm [15]. However, for some patients, mechanical removal of plaque via tooth-brushing is not sufficient [15, 16]. In a recent clinical practice guideline, developed by the European Federation of Periodontology (EFP)'s 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions participants and methodological consults, consensus was achieved on recommendations to manage gingival inflammation and risk factor control to prevent periodontal disease [10]. Supragingival dental biofilm control, professional mechanical plaque removal, and adjunctive therapies for gingival inflammation were all recommended by the workgroup [10]. Use of adjunctive locally delivered antimicrobials may also be appropriate for patients to reduce or eliminate subgingival biofilm [10].

Reviews of plaque-induced gingivitis management and treatment strategies find that chemical plaque control toothpaste significantly improves multiple measures of biofilm, plaque, and gingivitis [17, 18]. For some patients, removing plaque mechanically via tooth brushing is not sufficient to prevent more advanced disease [17, 18]. A recent meta-analysis to evaluate the efficacy of adjunctive therapies to reduce gingivitis and plaque found that, compared to mechanical removal of plaque alone, adjuncts improved gingival index, bleeding, and plaque index [18]. In a study of adult patients with periodontitis, adjunctive locally delivered antimicrobials improved plaque index compared to subgingival debridement alone [19]. Antiseptic agents reduced gingivitis and plaque in patients with plaque-induced gingivitis and patients with gingival inflammation [18]. Toothpastes containing stannous and amine have previously shown promising antibacterial properties [20, 21]. Zinc, naturally occurring in dental plaque, is frequently added as an antibacterial agent to oral health products [22, 23]. Because both amine and zinc have antimicrobial effects, together, they provide broader protection against oral bacteria. Further, amine-inclusive products have been shown to prevent biofilm development [24, 25] and a recent study of activated-zinc mouth rinse suggests that zinc reduces inflammation in gingival tissues [26].

In a previous clinical study employing a plaque regrowth model an amine and stannous fluoride system combined with zinc lactate in a mouthwash reduced plaque significantly within 3 days, compared to a negative control [27]. Most recently, Montesani et al. measured the antibacterial and clinical effectiveness of a novel formulation combining amine-zinc lactate-fluoride in a mouthwash and found significant improvements in plaque and gingivitis compared to a fluoride-only control [28].

The following study investigates the clinical efficacy of a new toothpaste containing amine, 0.5% zinc lactate and 1400 ppm F (NaF) to reduce plaque and gingivitis after 3 and 6 months of product use. The innovation of the test product is its new formulation: a combination of organic compound amine, with its surface active and antibacterial properties, and zinc, with its well-established antimicrobial and anti-inflammatory properties, and fluoride, with its demonstrated ability to remineralize enamel and prevent dental caries. Both site-level and patient-level analysis were conducted in a comparison of this new test toothpaste with a negative control toothpaste. An analysis on the number of inflamed sites brings insight to the BOP percentage experienced by subjects and a subject-level analysis brings new perspective to the overall performance of the amine + zinc + fluoride toothpaste from the patient perspective.

Materials and methods

Test products

The following were the test products of the investigation:

- Toothpaste containing amine compound, 0.5% zinc-lactate, and 1400 ppm F (NaF) (Test Group).
- Regular fluoride toothpaste containing 1450 ppm F as MFP/NaF (Negative Control Group).

Ethics approval

This study was performed in compliance with Good Clinical Practice (ICH-GCP), the Declaration of Helsinki and local legal and regulatory requirements. The study was reviewed and approved by the Institutional Review Board of Mahidol University, Thailand. No data sharing agreements were associated with this study. All data collected during the study were securely stored in compliance with ethical guidelines, with digital records protected through password-protected databases, and paper records stored in locked cabinets accessible only to study examiners.

Trial design and subjects

A randomized, double-blind, two-cell, parallel-group design on healthy adult male and female subjects from the Bangkok, Thailand area. The study location was the Mahidol University in Praves, Bangkok. All eligible subjects provided written informed consent.

Inclusion and exclusion criteria

Subjects were eligible for study inclusion if they were between the ages of 18 and 70 (inclusive), available for the study period, and met all the following criteria: in generally good health, had at least 20 uncrowned permanent natural teeth (excluding third molars), presented an initial gingivitis index of 1.0 or higher, as assessed by the Löe-Silness Gingival Index, and displayed an initial plaque index of at least 1.5, measured using the Quigley-Hein Plaque Index (Turesky Modification).

Subjects were ineligible for study inclusion if they met any of the following conditions: (1) presence of orthodontic bands, (2) presence of partial removable dentures, (3) soft or hard tissue tumors in the oral cavity, (4) advanced periodontal disease (characterized by purulent exudate, tooth mobility, or significant periodontal attachment or alveolar bone loss), (5) five or more decayed carious lesions requiring immediate treatment, (6) use of antibiotics within the month prior to study entry, (7) participation in another clinical study or test panel within the month prior to study entry, (8) a dental prophylaxis within the two weeks before baseline assessment, (9) known allergies to oral or personal care products or ingredients, (10) use of prescription medications that could interfere with study outcomes, (11) any medical condition preventing food or drink intake for up to 4 h, (12) history of alcohol or drug abuse, or (13) pregnancy or lactation.

All inclusion and exclusion criteria were checked by a questionnaire during the screening phase and at each visit.

Sample size calculation

To estimate sample size ($N = 100$; 50 per treatment arm), previous trials' attrition and other historical clinical data were considered. This data served as a reference point for the current study. Variability and distribution of the historical data helped in estimating key parameters such as the mean, standard deviation, and effect size. Sample size was calculated based on the standard deviation for the response measures (0.58), estimated attrition (10%), a 0.05 significance level, and a power level requirement of 80% (to detect a minimal statistically significant difference of 20%).

Randomization and blinding

Subjects were allocated to treatment groups according to a generated randomization. Subjects were assigned to one of two treatment groups using a randomization process based on computer-generated numbers. Drop-outs after randomization were not replaced. Together with the study products, the study investigator received a sealed envelope containing the randomization codes for each treatment group from the study sponsor. Had a medical emergency arose, the investigator could unblind a subject's treatment by opening the corresponding emergency decoding envelope. To maintain blinding, the sponsor applied unique codes and overlay packaging to each study product. Overlay packaging made test products visually similar. Neither the participants nor the researchers responsible for managing study procedures were aware of which group received which product.

Study interventions and time periods

Subjects were given a procedure, instructing them to brush twice daily (morning and evening) for two (2) minutes with their assigned toothpaste for a period of 6 months. Subjects were instructed to brush with a soft bristle adult toothbrush provided by the study investigators and were instructed to refrain from any other routine dental treatment. Procedure instructions were provided in writing to subjects. Oral soft and hard tissue assessments, as well as gingivitis and plaque evaluations were conducted at the baseline, 3-month and again at the 6-month examinations.

Gingivitis and plaque measurements

For each toothpaste group, gingivitis and dental plaque were assessed via the Löe-Silness gingival index and the Turesky Modification of the Quigley and Hein Plaque Index, respectively [29–31]. Details on measurement methods are reported elsewhere [28]. In short, gingivitis and dental plaque measurements were taken on each of the 6 scorable sides of the tooth and assigned a Loe-Silness gingival index score from 0 (no inflammation) to 3 (severe inflammation with tendency for spontaneous bleeding) [9], and Quigley-Hein plaque index score from 0 (no plaque) to 5 (plaque covering the majority of the crown of the tooth) by an examiner. Methods for calculating a whole mouth score, severity score, and interproximal indices were based on existing literature, and described by Montesani et al. 2024.

Adverse events

All adverse events or irregularities, whether reported by the subject or identified through examination of the hard and soft oral tissues, were documented from the initial screening visit through to the final visit.

Site-level and subject-level analyses

For a site-level analysis of gingivitis and plaque, a "healthy" site had a gingival index and plaque index score of 0 or 1, respectively. Sites were "unhealthy" with a gingival index score of 2 or 3 (inflamed) or a plaque index score of 2–5 (visible plaque). Sites with a baseline plaque score of 2–5 were further analyzed to measure improvement at each site over time. For the subject-level analysis of plaque, the percentage of subjects for each treatment who had ≥ 50% healthy sites for plaque index was calculated from the 3-month and 6-month examination measurements.

Statistical analyses

Statistical analyses were conducted separately for gingivitis and plaque evaluations. Gender differences between treatment groups were assessed with a Chi-Square test, while age differences were examined using an independent t-test. Treatment group comparisons for baseline gingival and plaque index scores were analyzed through analysis of variance (ANOVA), meaning, baseline differences and potential confounders were adjusted to isolate the effect of the independent variable on the dependent variable, while controlling for other variables that might influence the outcome. Changes within each treatment group, from baseline to follow-up in gingival and plaque index scores, were assessed using paired t-tests. For follow-up comparisons of treatment groups on baseline-adjusted gingival and plaque scores, analyses of covariance (ANCOVA) were applied. In ANCOVA, the baseline measurements were included as covariates in the model. By doing so, the analysis accounts for these initial differences, allowing for a more accurate comparison of the treatment effects.

Cohen's d, a standardized measure of effect size, was used in this study to quantify the difference between two group means in terms of standard deviations. The guideline for interpreting Cohen's d categorizes effect sizes as small (0.2), medium (0.5), and large (0.8). For instance, a Cohen's d value of 0.5 indicates that the two group means differ by 0.5 standard deviations. This implies that the average individual in one group is 0.5 standard deviations above the average individual in the other group.

All hypothesis tests were two-sided, with a significance level set at α=0.05.

Results

The study period was from January 18 to July 10, 2022. Recruitment and screening took place from January 13 to January 18, 2022.

Subject flow

Throughout the study, no adverse effects on the oral hard or soft tissues were reported. Of the 123 individuals assessed for eligibility, 23 did not meet eligibility criteria and 100 subjects were randomized into the test and negative control group. Eight (8) subjects were lost to follow-up and did not complete the study and dropped from the analysis (Fig. 1). Incomplete participation was not product-related. Forty-seven (47) and 45 test group and negative control group subjects were included in the analysis, respectively.

Baseline Data: Ninety-two (92) subjects completed the clinical study. The treatment arms of the study population did not differ significantly by gender or age (Table 1). Baseline measures for gingivitis and plaque did not differ significantly by any reported measure: index ($p = 0.632$ and 0.618, respectively), severity ($p = 0.675$ and 0.900, respectively), or interproximal ($p = 0.589$ and 0.568, respectively). The number of sites analyzed ($N = 10{,}788$) were 5,369 for the test group and 5,409 for the negative control group.

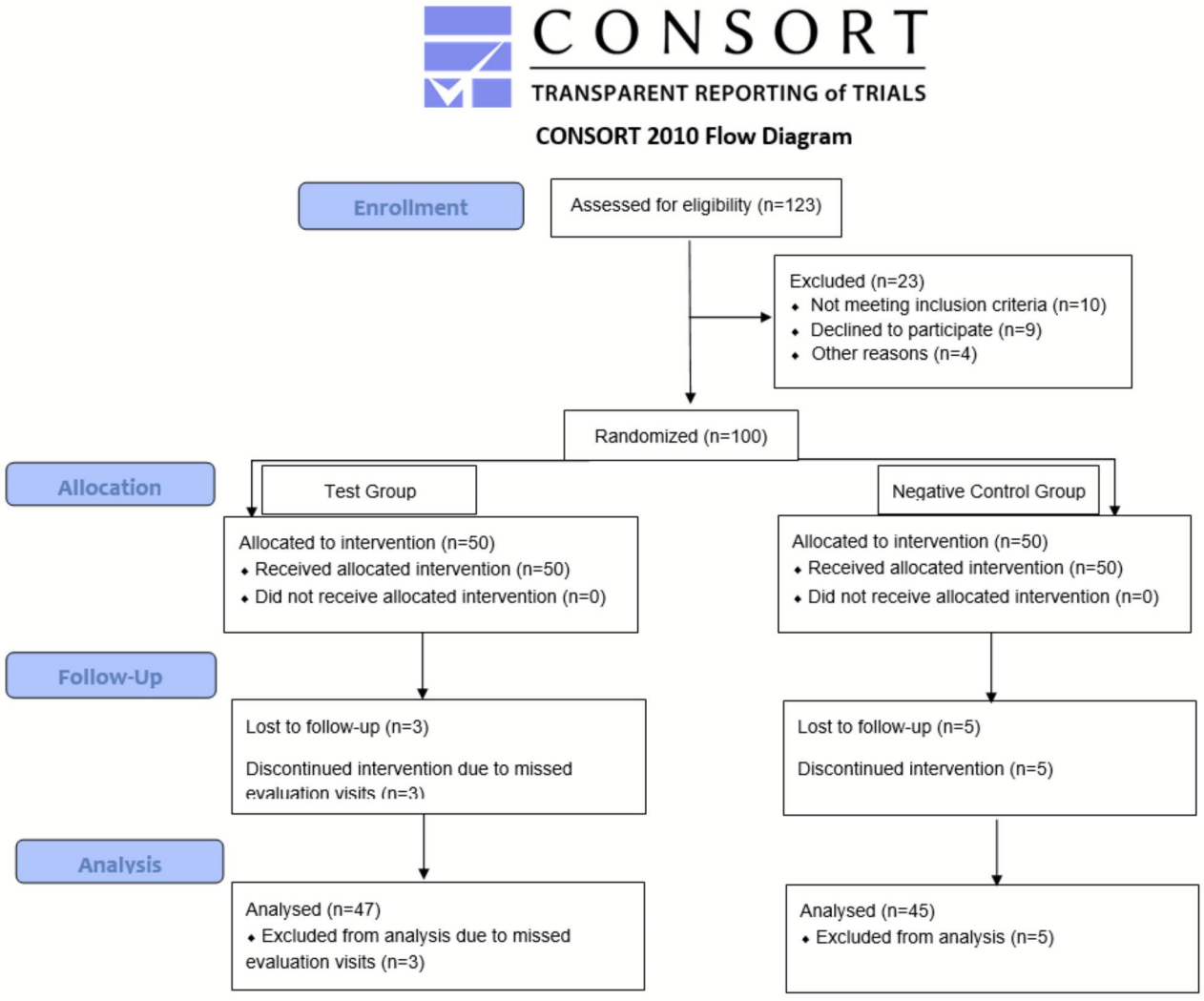

Fig. 1 Consolidated standards of reporting trials flow diagram of the study population for clinical evaluation. Figure 1 describes the flow of participants through the study, including enrollment, allocation, follow-up, and analysis. Of those assessed for eligibility ($N=123$), 23 were excluded and 50 were randomized into the test group and 50 were randomized to control. Among the test group, 3 were lost to follow up. Among the negative control group, 5 were lost to follow up. A total 92 subjects were included in the analysis

Clinical efficacy gingivitis

Table 2 provides a summary of the unadjusted mean values for gingival and plaque index scores, severity index scores, and interproximal scores recorded at baseline, 3-month, and 6-month time points for participants who completed the clinical study. Percent reductions from baseline values are also displayed. Both treatment groups showed statistically significant reductions in gingival and plaque indices, severity scores, and interproximal scores at the 3- and 6-month evaluations. However, the test group demonstrated considerably greater percent reductions across all measures compared to the negative control group (Table 2). Table 3 highlights comparisons of baseline-adjusted means for gingival and plaque index scores, severity scores, and interproximal scores, evaluated at baseline, 3 months, and 6 months for study completers. At both the 3-month and 6-month evaluations, the test group showed more substantial reductions in gingivitis and plaque than the control group (Table 3).

At the 3-month evaluation, reductions from baseline were significant ($p \leq 0.001$) for all gingival index, severity, and interproximal scores. Average reductions in gingival index from baseline were 19.0% for the test group and 3.1% for the control group (Table 2). Average gingival severity reductions were 36.2% for the test group and 4.2% for the control group (Table 2). For interproximal gingival scores, the reductions were 18.9% for the test group and 3.1% for the control group (Table 2).

By the 6-month evaluation, the most pronounced reductions were observed in gingival severity, with

Table 1 Summary of age and gender for subjects who completed the clinical study

Treatment	Number of Subjects			Age[3]	
	Male, *N*	Female, *N*	Total	Mean (S.D.)	Range
Test Group[1]	20	27	47	39.64 (8.52)	25–54
Negative Control Group[2]	23	22	45	37.60 (7.59)	26–56
All Treatment Groups	43	49	92	38.64 (8.10)	25–56

1. Toothpaste containing amine compound, 0.5% zinc lactate, and 1400 ppm F (NaF) (Colgate-Palmolive Co., New York, NY)

2. Toothpaste containing 1450 ppm F as MFP/NaF (Colgate-Palmolive Co., New York, NY)

3. No statistically significant difference was indicated between the two treatment groups with respect to gender ($p=0.411$) and age ($p=0.230$) characteristics

reductions of 49.3% ($p<0.001$) in the test group and 8.3% ($p<0.001$) in the control group (Table 2).

Following 3 months of treatment, these percentage differences indicated that the test toothpaste was 6 times more effective for gingivitis, 8 times more effective for gingival severity, and 6 times more effective for interproximal gingival scores compared to the negative control mouthwash. At the 6-month mark, the test toothpaste was found to be 5 times more effective across all measures.

At the 6-month evaluation, compared to the negative control group, the test group experienced statistically significant difference in improvement in gingival index scores (27.8%), gingival severity scores (42.2%; $p<0.001$), and gingival interproximal scores (28.0%; $p<0.001$). (See Table 3.)

Clinical efficacy plaque

For plaque measures, improvements from baseline were significant ($p<0.001$ for all measures). At the 3-month evaluation, the test and negative control groups experienced an average plaque index reduction from baseline of 16.3% and 1.8%, respectively (Table 2). For plaque severity, reductions from baseline at the 3-month evaluation were 19.4% and 2.7% for the test and negative control groups, respectively (Table 2). Lastly, for gingival

Table 2 Within-treatment comparisons of gingival and plaque index scores, severity scores, and interproximal

	Test group[1]			Negative control group[2]		
	Mean (SD)	Percent reduction from baseline	*p* value[3]	Mean (SD)	Percent reduction from baseline	*p* value[4]
Gingival index						
Baseline	1.89 (0.30)			1.93 (0.31)		
3 months	1.53 (0.30)	19.0%	$p<0.001$	1.87 (0.30)	3.1%	$p<0.001$
6 months	1.28 (0.30)	32.3%	$p<0.001$	1.82 (0.30)	5.7%	$p<0.001$
Gingival severity						
Baseline	0.69 (0.20)			0.72 (0.21)		
3 months	0.44 (0.22)	36.2%	$p<0.001$	0.69 (0.20)	4.2%	$p=0.001$
6 months	0.35 (0.17)	49.3%	$p<0.001$	0.66 (0.17)	8.3%	$p<0.001$
Gingival interproximal						
Baseline	1.90 (0.33)			1.96 (0.34)		
3 months	1.54 (0.31)	18.9%	$p<0.001$	1.90 (0.32)	3.1%	$p<0.001$
6 months	1.28 (0.31)	32.6%	$p<0.001$	1.84 (0.31)	6.1%	$p<0.001$
Plaque index						
Baseline	3.37 (0.66)			3.40 (0.68)		
3 months	2.82 (0.70)	16.3%	$p<0.001$	3.34 (0.69)	1.8%	$p<0.001$
6 months	2.32 (0.67)	31.2%	$p<0.001$	3.31 (0.69)	2.6%	$p<0.001$
Plaque severity						
Baseline	0.72 (0.21)			0.74 (0.21)		
3 months	0.58 (0.25)	19.4%	$p<0.001$	0.72 (0.21)	2.7%	$p=0.007$
6 months	0.41 (0.22)	43.1%	$p<0.001$	0.68 (0.22)	8.1%	$p<0.001$
Plaque interproximal						
Baseline	3.44 (0.69)			3.45 (0.70)		
3 months	2.85 (0.71)	17.2%	$p<0.001$	3.39 (0.70)	1.7%	$p<0.001$
6 months	2.34 (0.69)	32.0%	$p<0.001$	3.36 (0.70)	2.6%	$p<0.001$

1. Toothpaste containing amine compound, 0.5% zinc lactate and 1400 ppm F as NaF (Colgate-Palmolive Co., New York, NY)

2. Toothpaste containing 1450 ppm F as MFP/NaF (Colgate-Palmolive Co., New York, NY)

3. Significance of paired t-tests comparing the baseline and the 3-month examinations

4. Significance of paired t-tests comparing the baseline and the 6-month examinations

Table 3 Statistical parameters for comparisons made between treatment groups at 3-months and 6-month intervals, using baseline-adjusted means

	Mean (SD)		Percent difference[3]	*p* value[4]
	Test group[1]	Negative control group[2]		
Gingival Index				
3 months	1.54 (0.02)	1.85 (0.02)	16.8%	$p < 0.001$
6 months	1.30 (0.02)	1.80 (0.02)	27.8%	$p < 0.001$
Gingival Severity				
3 months	0.45 (0.01)	0.67 (0.02)	32.8%	$p < 0.001$
6 months	0.37 (0.01)	0.64 (0.01)	42.2%	$p < 0.001$
Gingival interproximal				
3 months	1.56 (0.02)	1.87 (0.02)	19.9%	$p < 0.001$
6 months	0.42 (0.01)	0.67 (0.01)	28.0%	$p < 0.001$
Plaque Index				
3 months	2.84 (0.02)	3.33 (0.02)	14.7%	$p < 0.001$
6 months	2.34 (0.02)	3.30 (0.02)	29.1%	$p < 0.001$
Plaque Severity				
3 months	0.59 (0.01)	0.71 (0.01)	16.9%	$p < 0.001$
6 months	0.42 (0.01)	0.67 (0.01)	37.3%	$p < 0.001$
Plaque interproximal				
3 months	2.86 (0.02)	3.38 (0.02)	15.4%	$p < 0.001$
6 months	2.35 (0.02)	3.35 (0.02)	29.9%	$p < 0.001$

1. Toothpaste containing amine compound, 0.5% zinc lactate and 1400 ppm F as NaF (Colgate-Palmolive Co., New York, NY)

2. Toothpaste containing 1450 ppm F as MFP/NaF (Colgate-Palmolive Co., New York, NY)

3. Difference between the baseline-adjusted means expressed as a percentage of the baseline-adjusted mean for the Negative Control Group. A positive value indicates a reduction in index scores for the Test Group relative to the Negative Control Group

4. Significance of ANCOVA comparison of baseline-adjusted means

interproximal, reductions from baseline for the test and negative control groups were 17.2% and 1.7%, respectively (Table 2).

At the 6-month evaluation, the average reduction of plaque index was 31.2% ($p < 0.001$) and 2.6% ($p < 0.001$) for the test and control groups, respectively (Table 2). At the 6-month evaluation, the greatest average reduction was seen in plaque severity: 43.1% ($p < 0.001$) and 8.1% ($p < 0.001$) for the test and control groups, respectively (Table 2).

After 3 months of treatment, these percentage differences resulted in the test toothpaste being 9× more effective for plaque index, 7× more effective in reducing plaque severity, and 10× more effective in reducing plaque interproximal index, compared to the negative control mouthwash. After 6 months of treatment, the test toothpaste was 12× more effective in reducing plaque, 5× more effective in reducing plaque severity, and 12× more effective in reducing plaque interproximal index compared to the negative control mouthwash.

Similarly, at the 6-month evaluation, compared to the negative control group, the test group experienced statistically significant difference in the improvement of the plaque index scores (29.1%; $p < 0.001$), plaque severity scores (37.3%; $p < 0.001$), and gingival interproximal scores (29.9%; $p < 0.001$).

Site-level analysis

Gingivitis

For those in the test group, subjects who had at least 50.0% healthy sites increased from 28 (59.6%) at 3 months to 35 (74.5%) at 6 months for the gingival index. Whereas, for the negative control group, only 9 subjects (20.0%) had at least 50.0% health sites at 3 months and that number remained unchanged at the 6-month evaluation.

Site-level analysis of the percentage of sites with improved gingival index scores among sites with moderate to severe inflammation at baseline (gingival index score of 2 or 3) is shown in Fig. 2. At baseline, the total number of inflamed sites (gingival index score of 2 or 3) as scored for gingivitis was 5,369 for the test group and 5,409 for the control group. For the test group, 48.9% and 62.9% of sites improved by at least 1 gingival index score from baseline at 3 months and 6 months, respectively (Fig. 2). For the negative control group, 15.4% and 23.4% of sites improved by at least 1 gingival index score from baseline at 3 months and 6 months, respectively (Fig. 2). This means that the test toothpaste was 2× more effective over the course of the study (6 months) than the negative control toothpaste to improve gingival index at the site-level.

Among the subgroup of sites with severe inflammation (gingival index of 3; number of sites = 1,589 for the test group and 1,564 for the negative control group), the percentages of sites with at least 1 gingival index score improvement were 59.4% at 3 months and 70.8% at 6 months for the test group and 22.4% at 3 months and 29.7% at 6 months for the negative control group. For those subjects with the most severe inflammation, the test toothpaste was 2× more effective over the course of the study (6 months) than the negative control toothpaste to improve gingival index at the site-level.

Plaque

At baseline, sites with severe plaque buildup (plaque index score ≥ 2) was 7,463 and 7,149 for test and negative control groups, respectively. For the test group, the number of sites that improved to healthy sites (plaque index score of 0 or 1) was 561 (7.5%) at 3 months and 1,579 (21.2%) at 6 months. For the negative control group, sites improving to healthy sites was 87 (1.2%) at 3 months and 259 (3.6%) at 6 months. This means that the test

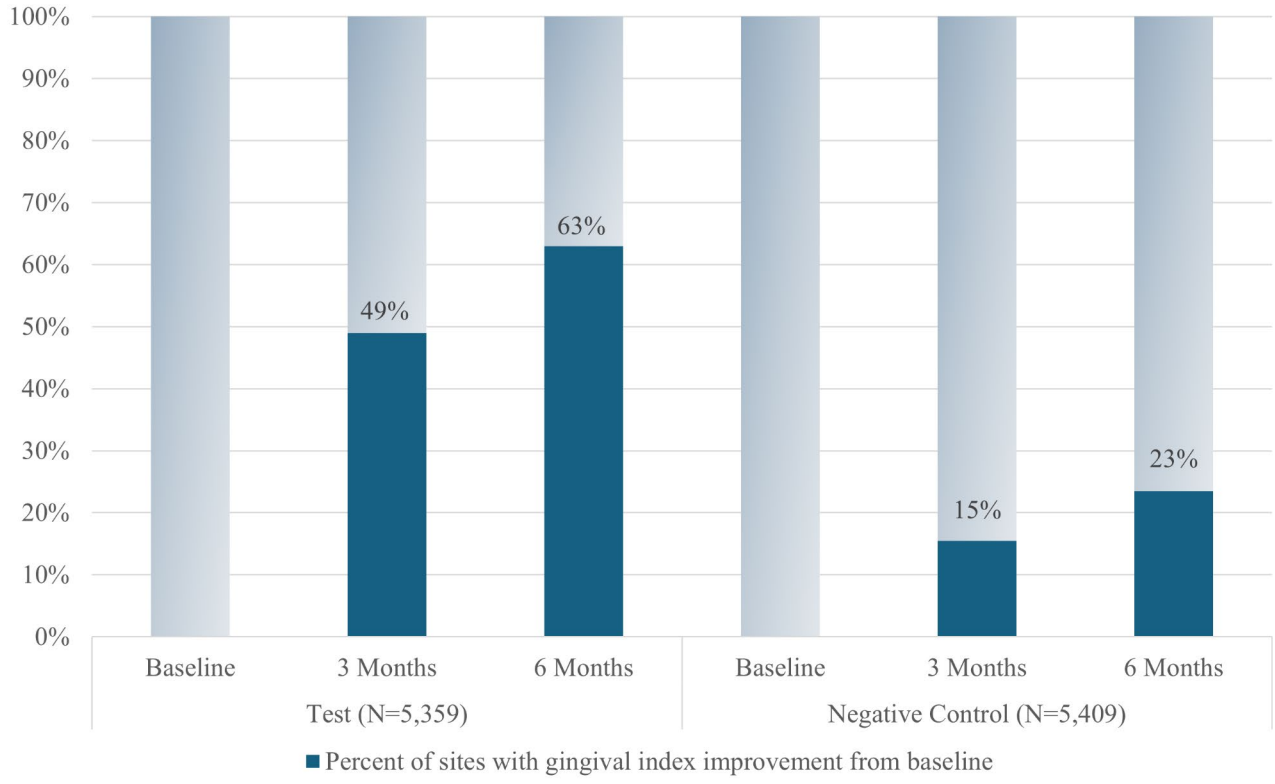

Fig. 2 Site-level improvement in gingival index scores among sites with moderate to severe inflammation at baseline. Figure 2 describes the site-level improvement of gingival index scores for those sites with moderate to severe inflammation (gingival index score of 2 or 3) at baseline. Improvement was defined as at least 1 gingival index score improvement from baseline. The percent of sites with improvement from baseline are reported. At 3 months, 49% of sites in the test group showed improvement, increasing to 65% at 6 months. In contrast, the negative control group showed improvement in 15% of sites at 3 months and 23% at 6 months

toothpaste was 5× more effective over the course of the study (6 months) than the negative control toothpaste to improve plaque at the site-level.

Safety results

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

Discussion

In this randomized controlled trial, a novel toothpaste combining amine, zinc lactate, and fluoride has shown beneficial clinical performance to the test subjects, making it suitable for consideration as part of the daily oral hygiene routine for patients with gingivitis. The results of this clinical study provide evidence regarding the daily use of amine + zinc + fluoride toothpaste to provide a significant reduction in gingivitis and plaque. For this study population, daily use led to an improvement in more than 60% of moderate to severely inflamed sites (gingival index of 2 or 3) after 6 months. For subjects with the most severe inflammation problems (gingival index of 3), more than 70% of sites improved after 6 months, meaning 7 of 10 sites with severe inflammation showed no bleeding after 6 months. Due to its antibacterial efficacy, amine + zinc + fluoride toothpaste was significantly more effective in reducing plaque and gingivitis in gingivitis patients after 3 and 6 months compared to a fluoridated negative control toothpaste.

The results reported here are congruent with previous studies indicating that an active ingredient toothpaste improves measures of gingivitis and plaque [17–19]. Toothpastes containing fluoride, amine, and/or zinc combat bacteria by disrupting bacterial metabolism and adhesion. Fluoride inhibits acid production by bacteria, while amine compounds destabilize bacterial cell walls, and zinc ions interfere with enzyme activity and prevent biofilm formation. A meta-analysis evaluating the efficacy of adjunctive therapies to reduce gingivitis and plaque found that, compared to mechanical removal of plaque alone, adjuncts chlorhexidine (CHX) 1%, triclosan, stannous fluoride/sodium hexametaphosphate improved gingival index, severity, and plaque index [18]. CHX (1%) and triclosan are not available in the form of toothpaste, so amine + zinc + fluoride serves as an alternative to stannous fluoride and hexametaphosphate toothpaste options.

Daily use of the test product aligns with the EFP recommendations: supragingival dental biofilm control,

professional mechanical plaque removal, and adjunctive therapies for gingival inflammation were all recommended by the workgroup [10].

Results reported here also align with Montesani et al.'s 2024 examination of an amine-zinc lactate-fluoride mouthwash [28]. In a comparison of baseline-adjusted means between test and a negative control fluoride mouthwash, the similar antibacterial system showed clinically meaningful improvement across plaque and gingival indices. These improvements were also seen in the current study and the percent improvements were especially similar among the plaque indices: The amine-zinc lactate-fluoride test mouthwash showed an improvement of 28% in plaque index, 58% in plaque severity, and 28% in plaque interproximal compared to the negative control mouthwash. For the current study, the same measures were 29%, 37%, and 30% improved compared to the negative control toothpaste.

This was a single site study in Bangkok, Thailand and conducted among subjects with indications of plaque and gingivitis, so the results may not be widely generalizable to other study populations. Future research conducted with a more heterogeneous study population and/or multiple sites would improve generalizability. Though subjects were given the same instructions for brushing, brushing techniques likely varied across subjects, changing the mechanical component of this study's exposure variable. This is why average, rather than individual, results are compared to controls. Similarly, variation by examiner has the potential to introduce bias and thus impacts outcome measures. Another limitation is the follow-up period. Subjects were followed for 6-months and results may vary over longer periods of time. Measurements were taken in a controlled trial environment so results may vary in a real-world setting. However, a strength of the study is incorporating multiple measures of gingivitis and plaque and, across all measures, the test group achieved better results than control with statistically significant reductions in the parameters characterizing plaque accumulation and subsequent gum problems. No safety concerns or adverse events, including any form of mucosal irritation or tooth staining were observed by the examiners in this study or reported by the subjects. The subjects who were enrolled but did not complete the study were lost to follow-up or discontinued use of the product for reasons unrelated to the treatment. It should be noted here that a standard clinical definition of gingivitis was developed in 2017 [4], so comparing the current research to past research is limited given the change in the clinical definition.

With the substantial healthcare costs [11] and patient burden associated with periodontal disease, coupled with the growing number of older patients affected [6], and evidence that poor oral health worsens health disparities [32], there is a clear need for accessible and affordable at-home treatments for gingivitis and plaque. The mechanical in-office removal of tartar and plaque, combined with the long-term use of an effective toothpaste for use at home, has been shown to help control the root cause of periodontal disease and improve gingival health [33, 34].

An upstream prevention strategy targeting gingivitis, caries, and periodontal disease can help reduce health disparities in oral health [34]. The test product demonstrates potential to lessen plaque accumulation and gingival inflammation, making it a potentially effective tool to prevent and treat gingivitis and ultimately slow progression to periodontal disease. Additionally, this approach has the potential to reduce healthcare costs associated with advanced gum disease.

However, a challenge of adherence to oral health routines may be rooted in a lack of awareness among patients regarding the advantage of using antibacterial toothpaste over regular toothpaste and thus educational initiatives for both patients and healthcare providers are necessary to highlight the importance of such products in maintaining oral health.

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Author contributions
D. S. managed the clinical trial on-site, recorded data, and communicated with the IRB committee, subjects, and site staff. T. T. served as the principal investigator, and managed the clinical trial on-site for clinical perspective. P. K. acted as the examiner. N. H. and T. P. prepared the study products and managed the shipments to the clinical site. L. M. conducted statistical analyses. M. S. was involved in discussions for study protocol design and results. Y. Z. was responsible for conceptualization, funding acquisition, supervision, and protocol design. All authors reviewed the manuscript and provided comments.

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

Declarations

Ethics approval and consent to participate
This study was performed in compliance with Good Clinical Practice (ICH-GCP), the Declaration of Helsinki and local legal and regulatory requirements. The study was reviewed and approved by the Institutional Review Board of Mahidol University, Thailand. All subjects signed an informed consent form.

Consent for publication
Not applicable.

Institutional review board approval
The study was reviewed and approved by the Institutional Review Board of DT/PY Mahidol University, 6 Yothi Street, Rajthevi, Bangkok, 10400, Thailand, telephone number: 662-200-7622, Certificate of Approval: COA.NO.MU-DT/PY-IRB 2021/ 100.3012.

Competing interests
Employment disclosure: Norbert Huber, Tilo Poth, Melissa Stellitano, and Yun-Po Zhang report employment with Colgate Palmolive Company. Funding disclosure: This study was supported by a grant by Colgate-Palmolive Company. Colgate-Palmolive commissioned the writing of this manuscript.

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