

## Research

# Efficacy of Flossing and Mouthrinsing Regimens on Plaque and Gingivitis: A randomized clinical trial

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### Abstract

**Purpose:** Flossing is a well-known component of daily recommended oral care regimens, but patients often find it challenging to perform effectively on a regular basis. The purpose of this 12-week supervised clinical trial was to investigate the effects of twice daily rinsing with a mouthrinse containing a fixed combination of four essential oils (4EO) and supervised daily dental flossing regimens as compared to a negative control 5% hydroalcohol rinse (NC) on the prevention and reduction of plaque, gingivitis, and gingival bleeding.

**Methods:** Volunteer participants who met the inclusion criteria were randomized into the following groups for the 12-week trial: 1) NC; 2) mouthrinse containing 4EO; 3) professional flossing performed by a dental hygienist (FBH); 4) supervised self-flossing (FUS). All participants received a professional dental prophylaxis prior to beginning the trial. On weekday mornings, all participants brushed on site. After brushing, the rinse groups used their products under supervision, and the floss groups had their teeth flossed by a dental hygienist or self-flossed under supervision. Participants performed their assigned regimen in the evenings and the twice-daily weekend use at home. Each individual assessment of oral hard and soft tissue, plaque, gingivitis, and gingival bleeding at weeks 4 and 12, probing depth and bleeding on probing at week 12 was made by the same calibrated examiner.

**Results:** Of 156 randomized participants, 149 completed the trial. Use of the 4EO mouthrinse statistically significantly reduced plaque, gingivitis, and gingival bleeding on probing after 12 weeks as compared to the NC rinse. Both flossing interventions statistically significantly reduced interproximal gingivitis and gingival bleeding at 12 weeks compared to the NC rinse; neither flossing intervention significantly reduced interproximal plaque after 12 weeks compared to the NC rinse.

**Conclusions:** Rinsing with a 4EO mouthrinse statistically significantly improved all oral health outcome measures at all time points compared to a NC rinse in this 12-week clinical trial. While professional and supervised flossing improved gingival health compared to use of the NC rinse, statistically significant plaque reduction with dental flossing was not attained at the end of the 12-week trial.

**Keywords:** dental plaque, gingivitis, essential oils, mouthrinse, chemotherapeutics, dental floss, oral health

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### Introduction

Dental biofilm (plaque), a complex community of microbial cells, attaches to the tooth surface by embedding in an extracellular matrix. Changes in the structure of the microbial communities within biofilm (plaque) serve as a primary etiologic factor in oral diseases such as caries and periodontitis.<sup>1</sup> Controlling plaque biofilm relies on a variety of methods and practices which include mechanical means

such as toothbrushing, as well as chemotherapeutics. Dental floss is classified by the Food and Drug Administration as a Class I medical device for removal of plaque and food particles between teeth to reduce tooth decay.<sup>2</sup> However, for many individuals, maintaining oral hygiene standards and mastering mechanical plaque control such as flossing, remains challenging.<sup>3</sup> Chemotherapeutic methods include

the use of toothpastes and mouthrinses to achieve plaque and gingivitis control. Numerous studies of six-month duration or longer and meta-analyses have demonstrated the safety and the efficacy in reducing plaque and gingivitis of a mouthrinse containing a fixed combination of four essential oils (4EO) (Listerine® Antiseptic Mouthwash; Johnson & Johnson Consumer Inc., Skillman, NJ, USA).<sup>4-11</sup>

In a systematic review conducted by Worthington et al., very low certainty of evidence was found for the efficacy of flossing, as an adjunct to toothbrushing, to reduce gingivitis over a one-to-six-month time frame.<sup>12</sup> In addition, there were inconsistent results among the studies included in the review and very low certainty of evidence in regard to the proportion of bleeding sites and plaque.<sup>12</sup> The Worthington review included two controlled studies conducted in unsupervised settings comparing 4EO and dental flossing in their ability to control accumulation of plaque and subsequently prevent/reduce gingivitis.<sup>12</sup> While unsupervised, in order to monitor compliance, both studies weighed the mouthrinse and floss on a monthly basis.<sup>13,14</sup> In the study by Barouth et al., it was shown that rinsing twice daily with 4EO was at least as good as daily flossing in reducing interproximal plaque and gingivitis.<sup>13</sup> The study by Sharma et al. showed that 4EO was at least as good as daily flossing in reducing interproximal gingivitis and significantly more effective than flossing in controlling interproximal plaque.<sup>14</sup>

As these studies were unsupervised, monitoring of the proper use of products and proper technique was not possible. Supervision during flossing studies is a method used to ensure correct use of product and proper technique. A review of the literature identified a lack of long-term supervised adult studies evaluating oral care regimens that included the use of floss for the reduction of plaque and gingivitis. Graves et al. compared the effectiveness of three types of dental floss and toothbrushing in reducing interproximal bleeding in a two-week supervised study.<sup>15</sup> While flossing, in combination with toothbrushing, was shown to be more effective than toothbrushing alone in reducing interproximal bleeding, the need for longer term clinical trials examining the efficacy of flossing was indicated.<sup>15</sup> The purpose of this 12-week supervised clinical trial was to investigate the effects of twice daily rinsing with a 4EO mouthrinse, as compared to professional and supervised flossing and the use of a negative control mouthrinse (5% hydroalcohol) on the reduction of plaque, gingivitis, and gingival bleeding.

## Methods

This randomized, controlled clinical trial was conducted at Salus Research, Inc. (Fort Wayne, IN, USA), an American Dental Association (ADA) qualified site,<sup>16</sup> from September 2018 to December 2018. The principles of the International Council on Harmonisation Guidance for Good Clinical Practice were applied to this trial. The trial protocol was approved by the Institutional Ethics Committee on research involving humans (IntegReview Institutional Review Board, Austin, TX, USA.) and was registered on clinicaltrials.gov (NCT04696536). In 2016, the ADA Council on Scientific Affairs modified the Seal of Acceptance program guidelines for chemotherapeutic products for control of gingivitis.<sup>17</sup> The revised clinical protocol guidelines indicate that the study duration length be a minimum of three months and include measurements at baseline and three months with the option of including an intermediate time point. Therefore, this clinical trial was three months in duration.

The randomization schedule was generated using a validated program created by the Biostatistics Department at Johnson & Johnson Consumer Inc. (JJCI). Participants were assigned in equal allocation to each treatment group using a block randomization with block size of eight. Each participant was assigned a unique randomization number that determined treatment assignment. The principal investigator (PI) and examiners were blinded to the treatment regimens of the participant groups. The personnel dispensing the test products or supervising their use did not participate in the examination of participants to minimize potential bias. Other staff members, including the PI and examiners, did not have access to the area where the product was being used.

## Sample

Participants were from the Fort Wayne, Indiana area and were selected for screening from the clinical test site's database based upon the following inclusion criteria: males and females in good general health over the age of 18 years, with no known allergies to commercial dental products, and at least 20 teeth with scorable facial and lingual surfaces. All participants needed to have evidence of gingivitis (although no minimum score on the Modified Gingival Index (MGI) was required), no evidence of severe periodontitis, and a minimum of 10 bleeding sites based on the Bleeding Index (BI).<sup>18,19</sup> Participants were eligible for the trial if they had no sites with >5 mm probing depth, and a maximum of three sites of 5 mm probing depth. Participants agreed to attend onsite (in the clinical setting) daily sessions on weekdays for study procedures. Other inclusion criteria included the

absence of fixed or removable orthodontic appliances or removable partial dentures and significant oral soft tissue pathology excluding plaque-induced gingivitis based on a clinical examination and discretion of the investigator/dental examiner. Female participants of childbearing potential were eligible if they had a negative pregnancy test and agreed to use medically acceptable methods of birth control for one month prior to the baseline evaluation and throughout the trial.

Exclusion criteria included dental prophylaxis within four weeks prior to baseline, needing antibiotics prior to dental treatment, use of certain medications within the last month (antibiotics, anti-inflammatory or anticoagulant therapy), use of chemotherapeutic oral care products within two weeks, being pregnant or lactating, use of smokeless tobacco, vaping or e-cigarettes or suspected substance abuse, and any other medical or psychiatric condition that would make the volunteer inappropriate for the trial in the judgment of the PI.

Participants were not permitted to have non-emergency dental procedures during the trial period. After receiving a thorough explanation of the trial and the opportunity to ask questions in private, all participants provided written informed consent on a document which complied with the requirements of the Health Insurance Portability and Accountability Act.

### **Interventions**

After receiving a dental prophylaxis, qualified participants were randomized into one of four treatment groups: 1) rinsing with a 5% hydroalcohol rinse (NC); 2) rinsing with an alcohol-containing product with a fixed combination of four essential oils: menthol, thymol, eucalyptol, and methyl salicylate (4EO); 3) professional flossing by a dental hygienist (FBH); and 4) self-flossing under supervision (FUS). All groups were required to brush with a fluoride dentifrice (Cavity Protection; Colgate-Palmolive, New York, NY, USA) prior to using their assigned regimen and were supplied with an ADA soft, flat-trim reference toothbrush sourced through the ADA. Participants assigned to FUS and FBH groups were instructed in a flossing method based on the ADA-recommended technique,<sup>20</sup> and were required to demonstrate competency. Those subjects assigned to FUS were observed daily by calibrated staff members and received reinforcement of flossing technique as needed throughout trial duration. The assigned products and materials for at-home use were provided to participants following clinical assessments. All trial products and materials were provided by the trial sponsor (Johnson & Johnson Consumer Inc., Skillman, NJ, USA).

Throughout the trial, all groups completed their first daily use of their assigned products/regimen under supervision at the trial site Monday through Friday. All groups brushed for one minute (timed) prior to proceeding with their assigned protocol. Participants assigned to the 4EO and NC groups rinsed for 30 seconds (timed) using 20ml of the assigned product. The professional flossing group (FBH) had their teeth flossed by a dental hygienist while the FUS group flossed under observation. Both groups used the same waxed floss product (REACH® Waxed Unflavored Dental Floss; JJCI, Skillman, NJ, USA.). Flossing was only performed once a day. Participants in both flossing groups flossed their own teeth on the weekends. All groups performed the second daily and weekend use of their assigned products unsupervised at home. Participants maintained diaries to document trial product use; diaries were reviewed, and the mouthrinse was weighed to track compliance at all assessment visits.

### **Assessments**

Participants were assessed at baseline, week 4, and week 12. Assessments at weeks 4 and 12 were made after the participants had refrained from using their assigned product for at least eight (but not more than 18) hours and not eating for at least four hours. All assessment visits included a review of inclusion/exclusion criteria and concomitant medications, oral examination of hard and soft tissues, and adverse event monitoring before other measurements were taken. Each clinical assessment was performed consistently throughout the trial by the same trained and calibrated clinical examiner. Calibration included annual intra-examiner repeatability exercises as part of the site's standard operating procedures.

The following assessments were conducted at baseline, week 4 and week 12: oral examination of hard and soft tissue, MGI, BI, probing depth and bleeding on probing (BOP, baseline and week 12 only), six-site Turesky modification of the Quigley-Hein Plaque Index (TPI) and Proximal Marginal Plaque Index (PMI).<sup>18,19,21-24</sup> The PMI was added to the assessments as an additional method for scoring plaque to corroborate the TPI results. All plaque assessments were supragingival measures. The primary efficacy endpoints were interproximal mean MGI and interproximal mean TPI at week 12. Secondary endpoints included interproximal mean MGI and interproximal mean TPI at week 4, whole mouth mean TPI and whole mouth mean MGI at weeks 4 and 12, whole mouth and interproximal mean BI and interproximal percent gingival bleeding sites at week 4, whole and interproximal mean BI and interproximal percent

gingival bleeding sites at weeks 4 and 12, and whole mouth and interproximal mean PMI at weeks 4 and 12. Exploratory endpoints were whole mouth and interproximal probing depth and BOP at week 12. Measurements were made at six sites for each graded tooth (mesiofacial, facial, distofacial, mesiolingual, lingual, distolingual). BOP measures were based on 1 = yes bleeding, 0 = no bleeding.

### ***Statistical analyses***

A sample size of 37 completed participants per group provides approximately 80% probability that the half-width for the confidence interval (CI) for the difference between two treatments is no more than 0.2, assuming a population standard deviation (SD) of 0.4, based on the historical database for MGI and TPI clinical trial data from the trial sponsor. This sample size also provides 90% power to detect a standardized effect size (difference between treatment means divided by SD) of at least 0.8. Sample sizes were estimated using PASS version 14.0.4 (NCSS Statistical Software, LLC, Kaysville, UT, USA).

Between-treatment efficacy comparisons were based on a mixed effects model for repeated measures analysis (MMRM), considering within-participants correlation as unstructured and with model terms for treatment and visit, and the corresponding baseline value as a covariate, including all participants with at least one assessment after baseline.<sup>25,26</sup> Treatment-by-visit and baseline-by-visit terms were included to make treatment comparisons and estimate treatment differences at specific visits. The 4EO and floss groups were compared for superiority to the NC group, with each comparison performed at the 0.05 level of significance, two-sided. Differences between 4EO and floss groups were assessed using 95% confidence intervals.

Comparisons between the 4EO and the flossing intervention groups were focused on estimation, specifically using point estimates and 95% confidence intervals, rather than hypothesis testing. This approach was taken due to the lack of previous information on long-term flossing as evaluated in this study, particularly the FBH group. However, a 95% confidence interval for the difference between 4EO and a floss group, amounts to a test of the null hypothesis that the two population means are equal at the 5% significance level, where the null hypothesis is rejected only if the interval does not contain zero.

Participant whole mouth mean MGI, whole mouth mean BI, and whole mouth mean TPI were calculated at baseline and each post-baseline assessment time point by taking the

mean of all observed scores at that time point. Interproximal means were calculated in the same way. Whole mouth percent gingival bleeding sites were calculated by taking the total number of sites with bleeding score >0 divided by the total number of sites assessed for each participant. Interproximal percent gingival bleeding sites were calculated in the same way but considering only interproximal sites. The interproximal mean for PMI was calculated similarly to the interproximal MGI, BI, and TPI. No imputation of missing data was performed.

For each of the secondary endpoints, the same MMRM approach, statistical testing and estimation procedures were applied. For the exploratory endpoints, each of which was assessed only at baseline and week 12 (therefore the MMRM approach was not applicable), the same treatment comparisons and confidence intervals were performed based on an analysis of covariance model with treatment as a factor and the corresponding baseline measure as a covariate. Demographic and baseline characteristics were compared across treatment groups using analysis of variance (ANOVA), Chi-square test, or Fisher's exact test.

## **Results**

Of the 156 randomized participants, 149 completed the trial. Four participants withdrew their consent and three were lost to follow-up. Trial group distribution is shown in Figure 1. The sample demographics and baseline gingival health characteristics are presented in Table I. Other than age, there were no significant differences among the groups for any other demographic data or for any average baseline data for all measurements.

### ***Interproximal Mean TPI and MGI***

As compared to the NC rinse group, the interproximal mean TPI was statistically significantly reduced for all treatments at week 4 (4EO: 29.5%; FBH: 11.7%; FUS: 6.73%), and for the 4EO group (22.8% reduction), but not the FBH (4.96%) or FUS (2.41%) groups, at week 12 (Table II). The interproximal mean MGI was statistically significantly reduced for 4EO (50.5% and 46.4%, respectively), FBH (26.0% and 26.4%, respectively) and FUS (18.6% and 21.6%, respectively) groups as compared to NC rinse at week 4 and week 12 (Table III).

### ***Interproximal Mean BI and Percent Bleeding Sites***

Interproximal mean BI was statistically significantly reduced for the 4EO group (59.0% and 76.4%, respectively), FBH group (67.8% and 85.6%, respectively) and FUS group



**Figure 1. Flow chart of trial group assignments (n=156)**

Study Groups					
	(NC) Mouthrinse	(4EO) Mouthrinse	Professional Flossing (FBH)	Supervised Flossing (FUS)	Totals
	n (%)	n (%)	n (%)	n (%)	n (%)
Randomized	39	40	37	40	156
Completed	36 (92.3)	40 (100.0)	35 (94.6)	38 (95.0)	149 (95.5)
Discontinued	3 (7.7)	0	2 (5.4)	2 (5.0)	7 (4.5)
Reason for Discontinuation					
• Withdrawal by Subject <sup>a</sup>	3 (7.7)	0	1 (2.7)	0	4 (2.6)
• Lost to follow-up	0	0	1 (2.7)	2 (5.0)	3 (1.9)
Safety Analysis Set	39 (100)	40 (100)	37 (100)	40 (100)	156 (100)
Full Analysis Set	37 (94.9)	40 (100)	36 (97.3)	39 (97.5)	152 (97.4)

a: subjects withdrew consent because they could not keep the daily schedule of on-site supervised use of assigned study products

(62.8% and 78.0%, respectively) compared to NC rinse group at weeks 4 and week 12 (Table IV). Likewise, interproximal percent bleeding sites were statistically significantly reduced for the 4EO group (58.4% and 78.5%, respectively), FBH group (68.9% and 86.0%, respectively) and FUS group (63.8% and 78.3%, respectively) as compared to NC rinse group at weeks 4 and 12 (Table V). All other secondary endpoints measured at week 12 are presented in Table IV and Table V.

### ***Interproximal Mean PMI***

Interproximal mean PMI at weeks 4 and 12 were largely directionally similar to interproximal mean TPI. At weeks 4 and 12 in comparison to the NC rinse group, the interproximal mean PMI was statistically significantly reduced for 4EO group by 54.9% and 50.0%, respectively, for the FBH group by 25.4% and 12.1%, respectively, and for the FUS group by 12.8% at week 4 only. Exploratory endpoints for whole mouth and interproximal probing depth and BOP at week 12 are presented in Table VI. All three

treatment groups statistically significantly reduced probing depth and BOP compared to the NC group.

### ***Interpretation of Differences Between 4EO and Flossing***

As noted in the statistical analysis description in the methods, comparisons between 4EO and floss groups were based on CIs, and statistical significance for 4EO versus floss groups can be assessed by whether the CIs contain 0 or not. Statistically significant reductions for the 4EO group vs each of the floss groups was observed for all endpoints based on MGI or TPI. For other endpoints, 4EO was in most cases not statistically significantly different versus either floss group. The only exception in favor of floss was that FBH statistically significantly reduced interproximal BOP at 12 weeks versus 4EO (Tables II-VI).

### ***Clinical safety***

The rinses and procedures were well tolerated by trial participants. Nineteen participants experienced at least one treatment-emergent adverse event (TEAE) during the study trial: four participants in the NC group, seven in the 4EO

**Table I. Demographics by group assignment (n=156)**

	NC Rinse	4EO Rinse	Professional flossing (FBH)	Supervised flossing (FUS)	Total n	p-value
n	39	40	37	40	156	
Mean age (SD)	39.3 (13.58)	38.2 (13.39)	44.6 (14.61)	33.0 (13.28)	38.6 (14.18)	0.004*
<b>Sex</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>0.392**</b>
Male	9 (23.1)	14 (35.0)	9 (24.3)	15 (37.5)	47 (30.1)	
Female	30 (76.9)	26 (65.0)	28 (75.7)	25 (62.5)	109 (69.9)	
<b>Race</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>0.750***</b>
White	33 (84.6)	33 (82.5)	30 (81.1)	28 (70.0)	124 (79.5)	
Black/African American	3 (7.7)	4 (10.0)	5 (13.5)	6 (15.0)	18 (11.5)	
Asian	1 (2.6)	1 (2.5)	—	2 (5.0)	4 (2.6)	
Native Hawaiian/Other Pacific Islander	—	—	1 (2.7)	—	1 (<1.0)	
American Indian/Alaskan Native	—	1 (2.5)	—	—	1 (<1.0)	
Other	2 (5.1)	1 (2.5)	1 (2.7)	4 (10.0)	8 (5.1)	
<b>Ethnicity</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>0.085***</b>
Hispanic/Latino	4 (10.3)	3 (7.5)	—	6 (15.0)	13 (8.3)	
Not Hispanic/Latino	35 (89.7)	37 (92.5)	37 (100)	34 (85.0)	143 (91.7)	
<b>Smoker</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>	<b>0.792***</b>
No	36 (92.3)	38 (95.0)	33 (89.2)	37 (92.5)	144 (92.3)	
Yes	3 (7.7)	2 (5.0)	4 (10.8)	3 (7.5)	12 (7.7)	
<b>Whole Mouth Baseline Scores</b>						
Mean MGI (SD)	2.07 (0.562)	2.17 (0.461)	1.99 (0.560)	2.15 (0.553)	2.10 (0.535)	0.433*
Mean TPI (SD)	3.01 (0.545)	3.07 (0.602)	2.83 (0.419)	3.04 (0.565)	2.99 (0.542)	0.208*
Mean BI (SD)	0.302 (0.1745)	0.306 (0.1989)	0.260 (0.1568)	0.343 (0.2496)	0.303 (0.1991)	0.350*
Mean % Bleeding Sites (SD)	21.17 (10.132)	20.64 (10.891)	18.11 (8.058)	23.11 (13.556)	20.81 (10.931)	0.254
Mean Pocket Depth (SD)	1.73 (0.208)	1.70 (0.197)	1.72 (0.275)	1.73 (0.265)	1.72 (0.236)	0.954*
<b>Interproximal Baseline Scores</b>						
Mean MGI (SD)	2.40 (0.491)	2.48 (0.373)	2.31 (0.488)	2.45 (0.467)	2.41 (0.457)	0.362*
Mean TPI (SD)	3.16 (0.486)	3.24 (0.543)	3.02 (0.366)	3.19 (0.500)	3.15 (0.482)	0.227*
Mean BI (SD)	0.315 (0.1837)	0.322 (0.2119)	0.276 (0.1975)	0.373 (0.2947)	0.322 (0.2273)	0.313*
Mean % Bleeding Sites (SD)	22.57 (11.057)	21.81 (11.610)	19.20 (11.118)	25.06 (16.166)	22.22 (12.755)	0.250
Mean PMI (SD)	3.20 (0.779)	3.38 (0.791)	3.08 (0.678)	3.26 (0.697)	3.23 (0.740)	0.332*

\*p-values are based on ANOVA model with term for treatment group.

\*\*p-values are based on Chi-Squares test.

\*\*\*Twenty percent or more cells with expected cell size &lt;5, Chi-Square test may not be valid test. Fisher's Exact test was used.

**Table II. Interproximal mean Turesky Plaque Index (TPI) at baseline, weeks 4 and 12**

	NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)
<b>Baseline</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
Mean (SD)	3.13 (0.453)	3.24 (0.543)	3.03 (0.368)	3.19 (0.506)
<b>Week 4</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
LSmean (SE)	3.09 (0.058)	2.17 (0.056)	2.72 (0.059)	2.88 (0.057)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	0.011
Difference (SE)		-0.91 (0.081)	-0.36 (0.083)	-0.21 (0.081)
95% CI		[-1.07, -0.75]	[-0.53, -0.20]	[-0.37, -0.05]
% reduction		29.5	11.7	6.7
<b>Treatment group versus FUS</b>				
Difference (SE)		-0.70 (0.079)		
95% CI		[-0.86, -0.55]		
<b>Treatment group versus FBH</b>				
Difference (SE)		-0.55 (0.082)		
95% CI		[-0.71, -0.39]		
<b>Week 12</b>				
<b>n</b>	<b>36</b>	<b>40</b>	<b>35</b>	<b>38</b>
LSmean (SE)	3.04 (0.056)	2.35 (0.053)	2.89 (0.057)	2.97 (0.054)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	0.060	0.347
Difference (SE)		-0.69 (0.077)	-0.15 (0.080)	-0.07 (0.078)
95% CI		[-0.85, -0.54]	[-0.31, 0.01]	[-0.23, 0.08]
% reduction		22.8	5.0	2.4
<b>Treatment group versus FUS</b>				
Difference (SE)		-0.62 (0.076)		
95% CI		[-0.77, -0.47]		
<b>Treatment group versus FBH</b>				
Difference (SE)		-0.54 (0.079)		
95% CI		[-0.70, -0.39]		

\**p*-values, model-based estimated means (LSmeans), and standard errors were based on a mixed effects model for repeated measures analysis (MMRM), with the fixed effects including treatment, visit, and treatment by visit interaction; baseline as a covariate; and baseline by visit interaction.

group, four in the FBH group and four in the FUS group. The types of TEAEs observed were: coated tongue (two in the NC group, five in the 4EO group, two in the FBH group and one in the FUS group); nausea (one in the NC group, one in the 4EO group and two in the FUS group); plicated tongue (one in the NC group, two in the 4EO group); toothache (one in the FUS group); headache (one each in the FBH and FUS groups) and one respiratory tract infection in the FBH group. Two incidences of coated tongue in the 4EO group were classified by the Investigator as possibly related to trial treatment. All TEAEs were mild to moderate severity and were documented and followed to resolution. No deaths or serious TEAEs were reported. No TEAEs resulted in participant withdrawal from the trial.

## Discussion

The purpose of this 12-week supervised clinical trial was to investigate the effects of twice daily rinsing with a mouthrinse containing a fixed combination of four essential oils (4EO) and supervised daily flossing regimens as compared to a negative control 5% hydro-alcohol rinse (NC) on the prevention and reduction of plaque, gingivitis, and gingival bleeding. Participants using the 4EO mouthrinse twice daily as part of their daily oral care regimen experienced statistically significant improvements in gingival health at all measurement points and in all assessments: reductions in plaque, gingivitis and gingival bleeding after four and 12 weeks, and probing depth and bleeding on probing after 12 weeks, as compared to the NC rinse. Investigation of

**Table III. Interproximal mean Modified Gingival Index (MGI) at baseline, weeks 4 and 12**

	NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)
<b>Baseline</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
Mean (SD)	2.37 (0.484)	2.48 (0.373)	2.31 (0.492)	2.44 (0.464)
<b>Week 4</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
LSmean (SE)	2.26 (0.073)	1.12 (0.071)	1.67 (0.075)	1.84 (0.072)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	<0.001
Difference (SE)		-1.14 (0.102)	-0.59 (0.105)	-0.42 (0.103)
95% CI		[-1.34, -0.94]	[-0.79, -0.38]	[-0.62, -0.22]
% reduction		50.5	26.0	18.6
<b>Treatment group versus FUS</b>				
Difference (SE)		-0.72 (0.100)		
95% CI		[-0.92, -0.52]		
<b>Treatment group versus FBH</b>				
Difference (SE)		-0.55 (0.103)		
95% CI		[-0.76, -0.35]		
<b>Week 12</b>				
<b>n</b>	<b>36</b>	<b>40</b>	<b>35</b>	<b>38</b>
LSmean (SE)	2.34 (0.070)	1.26 (0.067)	1.73 (0.071)	1.84 (0.068)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value		<0.001	<0.001	<0.001
Difference (SE)		-1.09 (0.097)	-0.62 (0.100)	-0.51 (0.098)
95% CI		[-1.28, -0.90]	[-0.82, -0.42]	[-0.70, -0.312]
% reduction		46.4	26.4	21.6
<b>Treatment group versus FUS</b>				
Difference (SE)		-0.58 (0.095)		
95% CI		[-0.77, -0.39]		
<b>Treatment group versus FBH</b>				
Difference (SE)		-0.47 (0.098)		
95% CI		[-0.66, -0.28]		

\**p*-values, model-based estimated means (LSmeans), and standard errors were based on a mixed effects model for repeated measures analysis (MMRM), with the fixed effects including treatment, visit, and treatment by visit interaction; baseline as a covariate; and baseline by visit interaction.

plaque accumulation and gingival inflammation employed multiple measures, focusing on interproximal sites as well as the whole mouth using plaque (TPI), gingivitis (MGI) and gingival bleeding indices (EBI). The TPI is a more universally utilized plaque index in clinical trials than the PMI but PMI produced a similar pattern in plaque reduction in comparison to TPI, helping to confirm robustness of the NC rinse findings. In this study, statistically significant reductions for the 4EO group versus each of the floss groups was observed at 4 and 12 weeks for MGI and TPI.

Comparing the mode of action of chemotherapeutic effects of 4EO on plaque and the mechanical disruption of plaque by floss may provide insight to these results. The essential oils of menthol, thymol, eucalyptol, and methyl salicylate have been shown to rapidly disrupt the bacterial cell wall through protein denaturation, bacterial enzyme activity alteration, bacterial endotoxin extraction, and increased bacterial regeneration time, resulting in a sustained reduction in bacteria regrowth over time.<sup>27</sup> Dental floss which is indicated for removal of plaque and food particles between teeth to reduce tooth decay, is able to remove interproximal plaque to some level.<sup>2,12</sup> In a classical clinical study on plaque biofilm growth and development, it was found that as early as 12 hours after rendering all tooth surfaces plaque-free, a consistent pattern of plaque development was evident, starting with the interproximal areas of the premolars and molars.<sup>28</sup> Based on these findings, Lang et al. theorized that the qualitative and quantitative bacterial composition in the saliva



**Table IV. Interproximal mean Bleeding Index (BI) at baseline, weeks 4 and 12**

	NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)
<b>Baseline</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
Mean (SD)	0.302 (0.1757)	0.322 (0.2119)	0.280 (0.1990)	0.373 (0.2986)
<b>Week 4</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
LSmean (SE)	0.316 (0.0204)	0.129 (0.0196)	0.102 (0.0207)	0.117 (0.0200)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	<0.001
Difference (SE)		-0.186 (0.0283)	-0.214 (0.0290)	-0.198 (0.0286)
95% CI		[-0.242, -0.130]	[-0.271, -0.157]	[-0.255, -0.141]
% reduction		59.0	67.8	62.8
<b>Treatment group versus FUS</b>				
Difference (SE)		0.012 (0.0280)		
95% CI		[-0.044, 0.067]		
<b>Treatment group versus FBH</b>				
Difference (SE)		0.028 (0.0285)		
95% CI		[-0.029, 0.084]		
<b>Week 12</b>				
<b>n</b>	<b>36</b>	<b>40</b>	<b>35</b>	<b>38</b>
LSmean (SE)	0.452 (0.0234)	0.107 (0.0223)	0.065 (0.0238)	0.099 (0.0229)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	<0.001
Difference (SE)		-0.346 (0.0323)	-0.387 (0.0333)	-0.353 (0.0328)
95% CI		[-0.409, -0.282]	[-0.453, -0.321]	[-0.418, -0.288]
% reduction		76.4	85.6	78.0
<b>Treatment group versus FUS</b>				
Difference (SE)		0.007 (0.0319)		
95% CI		[-0.056, 0.070]		
<b>Treatment group versus FBH</b>				
Difference (SE)		0.042 (0.0326)		
95% CI		[-0.023, 0.106]		

\**p*-values, model-based estimated means (LSmeans), and standard errors were based on a mixed effects model for repeated measures analysis (MMRM), with the fixed effects including treatment, visit, and treatment by visit interaction; baseline as a covariate; and baseline by visit interaction.

may have changed, subsequently influencing the rate of plaque accumulation.<sup>28</sup>

As discussed previously, the trial design included supervision of the daily use of dental floss as an adjunct to toothbrushing twice a day. Taking the flossing regimen further, it was either performed by a dental hygienist or by the participant who was monitored for proper use to investigate the role that effective flossing plays in reducing plaque and gingivitis. Both flossing intervention groups had statistically significant reductions in interproximal and whole mouth mean plaque scores as compared to the NC rinse group after four weeks, but not at 12 weeks. Participants in both flossing groups were found to have statistically significant improvements in their gingival health at all measurement points and in all assessments as compared to the NC rinse group in gingivitis and gingival bleeding at both four and 12 weeks.

Given the results with 4EO mouthrinse compared to the two flossing groups, the change in plaque composition from mechanical removal might not be as effective from a clinical perspective as from chemotherapeutic intervention. This may help explain some of the findings of this paper and warrants further investigation, including at the microbiome level, of a brush/floss/rinse routine in comparison to brushing alone, brush/floss, and brush/rinse routines.

There were improvements in probing depth and BOP after 12 weeks in the flossing groups. Two previous six-month unsupervised

**Table V. Interproximal percent gingival bleeding sites at baseline, weeks 4 and 12**

	NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)
<b>Baseline</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
Mean (SD)	21.83 (10.547)	21.81 (11.610)	19.39 (11.217)	24.96 (16.363)
<b>Week 4</b>				
<b>n</b>	<b>37</b>	<b>40</b>	<b>36</b>	<b>39</b>
LSmean (SE)	23.60 (1.374)	9.82 (1.321)	7.35 (1.400)	8.54 (1.348)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	<0.001
Difference (SE)		-13.78 (1.906)	-16.25 (1.961)	-15.06 (1.925)
95% CI		[-17.55, -10.02]	[-20.12, -12.37]	[-18.86, -11.25]
% reduction		58.4	68.9	63.8
<b>Treatment group versus FUS</b>				
Difference (SE)		1.27 (1.888)		
95% CI		[-2.46, 5.01]		
<b>Treatment group versus FBH</b>				
Difference (SE)		2.47 (1.924)		
95% CI		[-1.34, 6.27]		
<b>Week 12</b>				
<b>n</b>	<b>36</b>	<b>40</b>	<b>35</b>	<b>38</b>
LSmean (SE)	35.19 (1.478)	7.57 (1.406)	4.92 (1.506)	7.63 (1.445)
<b>Treatment group versus NC rinse</b>				
<i>p</i> -value*		<0.001	<0.001	<0.001
Difference (SE)		-27.62 (2.040)	-30.26 (2.110)	-27.55 (2.068)
95% CI		[-31.65, -23.59]	[-34.44, -26.09]	[-31.64, -23.47]
% reduction		78.5	86.0	78.3
<b>Treatment group versus FUS</b>				
Difference (SE)		-0.070 (2.017)		
95% CI		[-4.06, 3.92]		
<b>Treatment group versus FBH</b>				
Difference (SE)		2.64 (2.060)		
95% CI		[-1.43, 6.71]		

\**p*-values, model-based estimated means (LSmeans), and standard errors were based on a mixed effects model for repeated measures analysis (MMRM), with the fixed effects including treatment, visit, and treatment by visit interaction; baseline as a covariate; and baseline by visit interaction.

studies also found that the flossing groups had smaller observed reductions in interproximal plaque and gingivitis compared to the mouthrinse groups.<sup>13,14</sup> Similarly, a Cochrane review reported a very low certainty of evidence for the ability of flossing, when added to toothbrushing, to reduce gingivitis over a one to six-month time frame.<sup>12</sup> In the current trial, neither flossing group demonstrated a reduction in interproximal plaque compared to NC after 12 weeks indicating that flossing fails to prevent plaque build-up throughout the day.

An interesting finding was the BOP measurements in the FBH group which indicated that flossing by a dental hygienist resulted in a significantly greater mean reduction in interproximal BOP compared to the 4EO mouthrinse group. Additionally, the supervised flossing group also had directionally lower, but not statistically significant, mean interproximal BOP measurements as compared to the 4EO group. A potential explanation for this could be the deeper subgingival access and more thorough mechanical subgingival plaque disruption that effective flossing may provide as compared to the use of a 4EO mouthrinse. This study attempts to demonstrate the importance of technique in performing dental flossing for optimal results.

Oral health care providers are challenged with making patient care recommendations based on the unique needs of each individual. Results from this trial provide data-driven evidence to assist oral health care providers in recommending

Table VI. Whole-mouth, interproximal mean probing depth and bleeding on probing, week 12

			Whole Mouth Mean Probing Depth – Baseline			Interproximal Mean Probing Depth – Baseline			
	NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)		NC mouthrinse	4EO mouthrinse	Professional flossing (FBH)	Supervised flossing (FUS)
Mean (SD)	1.73 (0.202)	1.70 (0.197)	1.73 (0.276)	1.72 (0.268)	Mean (SD)	1.97 (0.238)	1.93 (0.231)	1.95 (0.320)	1.95 (0.304)
Week 12					Week 12				
n	37	40	36	39	n	37	40	36	39
LSmean (SE)	1.72 (0.012)	1.57 (0.012)	1.56 (0.012)	1.62 (0.012)	LSmean (SE)	1.97 (0.014)	1.78 (0.014)	1.76 (0.015)	1.83 (0.014)
Treatment group versus NC rinse					Treatment group versus NC rinse				
p-value*		<0.001	<0.001	<0.001	p-value*		<0.001	<0.001	<0.001
Difference (SE)		-0.15 (0.017)	-0.17 (0.017)	-0.11 (0.017)	Difference (SE)		-0.18 (0.020)	-0.21 (0.021)	-0.13 (0.020)
95% CI		[-0.19, -0.12]	[-0.20, -0.13]	[-0.14, -0.07]	95% CI		[-0.22, -0.14]	[-0.25, -0.17]	[-0.17, -0.09]
% reduction		8.9	9.6	6.1	% reduction		9.35	10.6	6.7
Treatment group versus FUS					Treatment group versus FUS				
Difference (SE)		-0.05 (0.017)		1	Difference (SE)		-0.05 (0.020)		
95% CI		[-0.08, -0.02]			95% CI		[-0.09, -0.01]		
Treatment group versus FBH					Treatment group versus FBH				
Difference (SE)		0.01 (0.02)			Difference (SE)		0.02 (0.020)		
95% CI		[-0.02, 0.05]			95% CI		[-0.01, 0.06]		
Whole Mouth Mean Bleeding on Probing – Baseline					Interproximal Mean Bleeding on Probing – Baseline				
n	37	40	36	39	n	37	40	36	39
Mean (SD)	0.314 (0.1475)	0.324 (0.1712)	0.303 (0.1391)	0.347 (0.1827)	Mean (SD)	0.352 (0.1778)	0.361 (0.1931)	0.346 (0.1564)	0.386 (0.1983)
Week 12					Week 12				
n	36	40	35	38	n	36	40	35	38
LSmean (SE)	0.349 (0.0168)	0.224 (0.0159)	0.182 (0.0170)	0.218 (0.0163)	LSmean (SE)	0.389 (0.0196)	0.261 (0.0186)	0.182 (0.0199)	0.228 (0.0191)
Treatment group versus NC rinse					Treatment group versus NC rinse				
p-value*		<0.001	<0.001	<0.001	p-value*		<0.001	<0.001	<0.001
Difference (SE)		-0.125 (0.0231)	-0.168 (0.0239)	-0.131 (0.0234)	Difference (SE)		-0.128 (0.0270)	-0.207 (0.0279)	-0.161 (0.0273)
95% CI		[-0.171, -0.079]	[-0.215, -0.120]	[-0.177, -0.085]	95% CI		[-0.181, -0.074]	[-0.262, -0.152]	[-0.215, -0.107]
% reduction		35.8	48.0	37.5	% reduction		32.9	53.2	41.5
Treatment group versus FUS					Treatment group versus FUS				
Difference (SE)		0.006 (0.0228)			Difference (SE)		0.033 (0.0266)		
95% CI		[-0.039, 0.051]			95% CI		[-0.019, 0.086]		
Treatment group versus FBH					Treatment group versus FBH				
Difference (SE)		0.043 (0.0233)			Difference (SE)		0.079 (0.0272)		
95% CI		[-0.003, 0.089]			95% CI		[0.025, 0.133]		

\*p-values, model-based estimated means (LSmeans), and standard errors were based on analysis of covariate with term for treatment and baseline as a covariate.

effective plaque and gingivitis control methods as part of their patients' customized oral care regimens. Adding an easy-to-use intervention such as a 4EO mouthrinse to a patient's oral care routine provides an effective option to manage gingivitis and supragingival plaque accumulation.

### Limitations

The sample population was limited to people who volunteered to be part of a clinical trial at a research center in the Midwest and may not be representative of the general population. The inclusion criteria specifically recruited people with evidence of gingivitis and without evidence of severe periodontitis and the results may not be generalizable to a population with more optimal oral health or with greater disease. This trial did not address the possible differences in efficacy of a three-step routine of brushing, flossing, and rinsing as compared to a two-step routine of brushing and rinsing under supervision nor did it evaluate the toothbrushing technique. The trial only investigated flossing once a day rather than multiple occasions daily. Furthermore, floss was the only interdental cleaning device that was investigated in this trial. Future research should include various combinations of mechanical and chemotherapeutic agents.

### Conclusions

Twice daily use of a mouthrinse containing four essential oils, menthol, thymol, eucalyptol and methyl salicylate, combined with twice daily toothbrushing statistically significantly reduced plaque, gingivitis and gingival bleeding at 4 and 12 weeks as compared to a 5% hydroalcohol negative control rinse. Both the professional flossing (FBH) and the supervised self-flossing (FUS) groups demonstrated improved gingival health measures as compared to the negative control rinse group. Statistically significant plaque reduction in the flossing groups was attained at week 4 but not at week 12.

### Disclosures

Johnson & Johnson Consumer Inc., (JJCI; Skillman, NJ, USA) sponsored this clinical trial and was responsible for the trial design and the collection, analysis, and interpretation of the data. Mary Lynn Bosma, James McGuire, Anusha Sunkara, and Pamela Sullivan are employees of JJCI. Jeffery Milleman and Kimberly Milleman are principals at Salus Research, Inc, Fort Wayne, IN, USA and received grants from JJCI and conducted the trial on behalf of JJCI. Abbie Yoder is an employee of Salus Research, Inc.

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### References

1. Valm AM. The structure of dental plaque microbial communities in the transition from health to dental caries and periodontal disease. *J Mol Biol*. 2019 Jul 26;431(16):2957-69.
2. Food and Drug Administration. Code of Federal Regulations Title 21: Dental floss [Internet]. Washington DC: Department of Health and Human Services; 2022 [cited 2022 Mar 1]. Available from: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRsearch.cfm?FR=872.6390>
3. Marsh PD. Contemporary perspective on plaque control. *Brit Dent J*. 2012 Jun;212(12):601-6.
4. Araujo MWB, Charles CA, Weinstein RB, et al. Meta-analysis of the effect of an essential oil-containing mouthrinse on gingivitis and plaque. *J Am Dent Assoc*. 2015 Aug;146(8):610-22.
5. Tilliss T, Carey CM. As an adjunct to mechanical oral hygiene, essential oil-containing mouthwash, compared to floss, cetylpyridinium chloride, or placebo may be more effective in reduction of plaque and gingivitis. *J Evid Base Dent Pract*. 2017 Sept;17(3):256-8.

6. Haas AN, Wagner TP, Muniz FWMG, et al. Essential oils-containing mouthwashes for gingivitis and plaque: Meta-analyses and meta-regression. *J Dent*. 2016 Dec;55:7-15.
7. Van Leeuwen MP, Slot DE, Van der Weijden, GA. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: A systematic review and meta-analysis. *Int J Dent Hyg*. 2014 Aug;12(3):160-7.
8. Charles CA, Lisante TA, Revankar R, et al. Early benefits with daily rinsing on gingival health improvements with an essential oil mouthrinse--post-hoc analysis of 5 clinical trials. *J Dent Hyg*. 2014 Jan;88(Suppl 1):40-50.
9. Boyle P, Koechlin A, Autier P. A systematic review with meta-analysis of mouthwash use for the prevention of dental plaque. *Oral Dis*. 2014 Jan;20(Suppl 1):17-21.
10. Neely AL. Essential oil mouthwash (EOMW) may be equivalent to chlorhexidine (CHX) for long-term control of gingival inflammation but CHX appears to perform better than EOMW in plaque control. *J Evid Base Dent Pract*. 2011 Dec;11(4):171-4.
11. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *J Am Dent Assoc*. 2006 Dec;137(12):1649-57.
12. Worthington HV, MacDonald L, Pericic PT, et al. Home use of interdental cleaning devices, in addition to toothbrushing, for preventing and controlling periodontal diseases and dental caries. *Cochrane Database Sys Rev*. 2019 Apr10;4(4) CD012018.
13. Bauroth K, Charles CH, Mankodi SM, et al. The efficacy of an essential oil antiseptic mouthrinse vs. dental floss in controlling interproximal gingivitis: A comparative study. *J Am Dent Assoc*. 2003 Mar;134(3):359-65.
14. Sharma NC, Charles CH, Qaqish JG, et al. Comparative effectiveness of an essential oil mouthrinse and dental floss in controlling interproximal gingivitis and plaque. *Am J Dent Assoc*. 2002 Dec;15(6):351-5.
15. Graves RC, Disney JA, Stamm JW. Comparative effectiveness of flossing and brushing in reducing interproximal bleeding. *J Periodontol*. 1989 May;60(5):243-7.
16. American Dental Association. ADA Seal of Acceptance: independent research testing site qualification [Internet]. Chicago (IL): American Dental Association; 2022 [cited 2022 Mar 1] [about 6 screens]. Available from: <https://www.ada.org/resources/research/science-and-research-institute/ada-seal-of-acceptance/ada-seal-resources/ada-seal-testing-sites>
17. ADA Council on Scientific Affairs. Acceptance program requirements. Chemotherapeutic products for control of gingivitis. Chicago (IL): American Dental Association; Jun 2016. 10 p.
18. Lobene RR, Weatherford T, Ross NM, et al. A modified gingival index for use in clinical trials. *Clin Prev Dent*. 1986 Jan-Feb;8(1):3-6.
19. Saxton CA, van der Ouderaa FJ. The effect of a dentifrice containing zinc citrate and Triclosan on developing gingivitis. *J Periodontal Res*. 1989 Jan;24(1):75-80.
20. American Dental Association. How to floss [Internet]. Chicago (IL): American Dental Association; 2012 [cited 2022 Mar 1]. Available from [https://www.mouthhealthy.org/-/media/MouthHealthy/Files/Kids\\_Section/ADAHoToFloss\\_Eng.pdf?la=en](https://www.mouthhealthy.org/-/media/MouthHealthy/Files/Kids_Section/ADAHoToFloss_Eng.pdf?la=en).
21. Lobene RR, Soparkar PM, Newman MB. Use of dental floss. Effect on plaque and gingivitis. *Clin Prev Dent*. 1982 Jan-Feb;4(1):5-8.
22. Newman MG, Takei H, Klokkevold PR, Carranza FA. Newman and Carranza's clinical periodontology. 13th ed. Philadelphia (PA): Elsevier; 2018 May. 944 p.
23. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of Vitamin C. *J Periodontol*. 1970 Jan;41(1):41-3.
24. Benson BJ, Henyon G, Grossman E, et al. Development and verification of the proximal/marginal plaque index. *J Clin Dent*. 1993;4(1):14-20.
25. Mallinckrodt CH, Clark WS, David SR. Accounting for dropout bias using mixed-effects models. *J Biopharm Stats*. 2001 Feb-May;11(1-2): 9-21.
26. Siddiqui O, Hung HMJ, O'Neill R. MMRM vs LOCF: a comprehensive comparison based on simulation study and 25 NDA datasets. *J Biopharm Stats*. 2009 Feb;19(2):227-46.
27. Fine DH, Letizia J, Mandel ID. The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. *J Clin Periodontol*. 1985 Sep;12(8):660-6.
28. Lang NP, Cumming BR, Loe H. Toothbrushing frequency as it relates to plaque development and gingival health. *J Periodontol*. 1973 Jul;44(7):396-405.