Clinical Oral Investigations
https://doi.org/10.1007/s00784-021-03881-0

ORIGINAL ARTICLE

Reductions in clinical inflammation and oral neutrophils with improving oral hygiene

Prem K. Sreenivasan[1,2] · Prasad K.V.V[3] · Shweta Sharda[3] · Yogitha Pothamsetty[3]

Received: 21 December 2020 / Accepted: 10 March 2021

Abstract

Objective This study compared the effects of oral hygiene with a toothpaste formulated with zinc (test) to a fluoride dentifrice (control) for effects on oral polymorphonuclear leukocyte (PMN) as a measure of whole mouth inflammation along with effects on clinical parameters of dental plaque and gingivitis.

Materials and methods Adults (age range 18–60 years, $n = 212$) completed this double-blind, parallel design study. After washout, a baseline oral rinse sample was evaluated for PMN prior to clinical assessments for gingivitis and dental plaque. Subjects were randomly assigned to brush twice daily with either the test or the control toothpaste. Post-treatment evaluations repeated all baseline assessments after 4-week, 6-week and 12-week use of dentifrice with all assessments conducted 12 hours after brushing.

Results PMN reductions in the test were 16.8%, 18.7% and 42.5% at the 4-week, 6-week and 12-week evaluations respectively and significantly different from the control ($p < 0.05$). The test toothpaste also demonstrated progressively increasing reductions in gingivitis and dental plaque that ranged from 7.6 to 33.3% and 2.3 to 9.1% respectively versus the control ($p < 0.05$).

Conclusions The test dentifrice demonstrated progressive reductions in oral PMN representing whole mouth inflammation in conjunction with improvements in oral hygiene as compared to the control toothpaste.

Clinical relevance A hallmark of oral inflammation includes the accumulations of PMN in the afflicted gingival regions to reduce the influences of proliferating microorganisms. Brushing with a zinc dentifrice demonstrated progressive reductions in oral PMN and improvements in oral hygiene as evidenced by progressively lower dental plaque and gingival indices.

Keywords Dental plaque · Gingivitis · Neutrophils · Zinc salts

Introduction

A diverse polymicrobial microflora is routinely isolated from distinct ecological niches of the human mouth [38]. Endogenous oral organisms include biofilms on the surfaces of hard and soft tissues and as non-adherent salivary components suggesting potential niche preferences based on localization. Commencing with initial reports from the 1960s, clinical studies demonstrate associations between these endogenous organisms and the initiation of oral diseases [4, 21]. These studies report an increasing proportion of gram-negative organisms within the dental plaque in the absence of routine oral hygiene during the transition from health to gingival inflammation. Reported in the literature are new organisms in halitosis [16], caries [2] and other inflammatory conditions of the periodontium [28, 29].

Analysis of the complex oral microbial ecosystem reveals metabolic aspects unique to these organisms. Complex microbial metabolites, a feature of these organisms and their inflammatory components, such as lipopolysaccharide, are readily isolated during the transition from health to gingivitis [18] and trigger specific host responses. Professional dental care and effective routine mechanical oral hygiene are primary steps in the control of bacterial flora and the associated inflammation [44]. While effective self-care is emphasized and widely recommended by dental professionals, global surveys consistently report inadequate hygiene in most populations [4]. Almost 50% of the plaque is left behind on the teeth following an

✉ Prasad K.V.V
kakarlap@hotmail.com

1 HITLAB, 3960 Broadway, New York, NY 10032, USA

2 Department of Oral Biology, Rutgers School of Dental Medicine, Newark, NJ 07103, USA

3 Department of Public Health Dentistry, SDM College of Dental Sciences and Hospital, Affiliated to SDM University, Dharwad, India

Published online: 19 March 2021

episode of tooth brushing [8] with gingivitis, an inflammatory condition, reported from all populations [21]. Approaches to supplement oral hygiene procedures include the routine use of formulations with antimicrobial agents. A dentifrice formulated with zinc salts has been a subject of clinical studies, with results demonstrating reductions in dental plaque and associated gingivitis [9], and significant reductions in the microbial flora of distinct oral niches [39] all of which represent improvements in oral health.

Inflammation of oral tissue is uniformly characterized by histological features reporting a rapid infiltration of neutrophils through the inflamed vessels of afflicted regions [11]. Polymorphonuclear neutrophils (PMN) are the most abundant leukocyte population during inflammatory conditions of the mouth [18] with a range of protective functions [31] and reportedly form a barrier between bacterial plaque and the gingival tissue to wall off the growing microorganisms. Following extravasation, neutrophils accumulate in oral fluids with approximately 80% retaining viability for a short duration [11]. These observations have an extended history including histologic and functional characterizations [33]. Studies report non-invasive assays to evaluate oral neutrophils in health, periodontal disease and other conditions [1, 5, 31, 45]. We hypothesized that the effects of an intervention that can reduce gingivitis may be evaluated by enumerating oral PMN. Accordingly, the present clinical investigation examined the effects of oral hygiene with the described zinc toothpaste on oral neutrophils amongst adult subjects in conjunction with clinical parameters of dental plaque and gingivitis.

Materials and methods

Study population and experimental design

This study was designed as a double-blind, parallel design, single-center trial to evaluate the outcomes after 12-week use of randomly assigned toothpastes on oral PMN and clinical outcomes. The study was approved by the Institutional Ethics Committee of the SDM College of Dental Sciences and Hospital and conducted in accordance with widely accepted practices for clinical studies. Subjects from the local area who provided voluntary written informed consent were scheduled for a screening visit at the dental clinic to determine eligibility for study inclusion. The study is reported in accordance with the CONSORT statement.

Subject inclusion criteria

Prospective subjects of either gender in good health (age range 18–70 years) from the local area were scheduled for an initial screening visit. A dentist conducted a whole mouth clinical examination during the screening visit with all assessments conducted in the morning in the dental operatory under standard lighting. Enrolled subjects had at least 20 natural teeth, and did not have removable or fixed dental prostheses. Study enrollment was restricted to those registering a minimum dental plaque index score of 1.5 by the Turesky modification of the Quigley-Hein Index [42] and a minimum gingivitis index score of 1.0 by the Loe-Silness Index [22].

Subject exclusion criteria

Individuals reporting pregnancy or anticipating pregnancy were excluded. Subjects reporting chronic or serious conditions, such as diabetes mellitus, heart disease or renal and liver disease; or transmissible conditions, e.g. AIDS; or taking prescription medications, were excluded. Also excluded were those presenting oral conditions that required dental care such as ulcers, abscess, carious lesions, restorations including reported allergy of oral hygiene formulations, concurrent participation in another clinical study, participation in a clinical study in the preceding month or a history of tobacco use.

Following enrollment, subjects were scheduled for baseline clinical evaluations as described below.

Clinical procedures

Baseline and test product assignment

Enrolled subjects underwent a 1-week washout phase with a commercially available fluoride toothpaste and a soft-bristled toothbrush with a twice-daily oral hygiene regime. Study participants were instructed to discontinue the use of all other oral hygiene formulations for the remainder of the study period.

After the washout phase, subjects were scheduled to arrive at the dental clinic for baseline evaluations. Subjects refrained from oral hygiene for 12 hours, prior to their visit and all examinations were conducted in the morning. At the baseline visit, subjects were provided 10 ml of sterile saline solution. Subjects were instructed to rinse their mouth for 10 s and expectorate into a sterile tube for neutrophil assessments (PMN) with these steps representing minor modifications from those reported previously [1, 5, 45]. All subjects underwent clinical assessments for plaque and gingival indices by one calibrated examiner. The clinical examiner was calibrated in exercises prior to the start of the study with a kappa score of 0.9 recorded in the evaluations. After completing the baseline assessments, subjects were randomly assigned either a fluoride toothpaste (control) or a fluoride toothpaste formulated with zinc salts (test) with all dentifrices overwrapped to mask all identifying features and assigned a unique code to blind the subjects and examiners for treatment assignments. Subjects completed the first use of their assigned toothpaste in the dental clinic and instructed to brush twice

daily with the provided toothpaste. No other brushing instructions were provided and subjects instructed to maintain their usual eating and dietary habits but instructed to refrain from using any other oral hygiene formulations over the 12-week study period.

Post-treatment evaluations

Subjects were scheduled to return for post-treatment assessments similar to baseline that were conducted after 4-week, 6-week and 12-week use of provided toothpaste. Study personnel interviewed subjects during each recall visit for adverse events and reminded subjects of study procedures to emphasize compliance with study procedures. During the study, subjects were periodically contacted to reinforce study procedures. At the conclusion of the study, all issued toothpastes were collected and weighed as an additional measure of study compliance and subjects provided their compensation.

Laboratory procedures for PMN quantitation Procedures for PMN quantitation were similar to those described previously and utilized fluorescent microscopy [1, 5, 45]. In brief, all samples were transported to the laboratory and mixed gently with formalin. Samples were stored at 4 °C and stained with ethidium bromide for microscopy. Microscopic results were reported as number of PMN per ml of sample.

Statistical methods

Sample-size calculations for this study estimated inclusion of approximately 100 subjects in each treatment group to detect a difference of approximately 0.2 units between treatments with an attrition rate of 5%. Calculations were based on standard deviation values of 0.5 units with α of 0.05 and statistical power of 80%.

Statistical analyses

Demographic variables between treatment groups were compared by a chi-square analysis with an analysis of variance (ANOVA) comparing the age of enrolled subjects. PMN counts were transformed ($\log_{10}$) for analysis. PMN and clinical results recorded over the course of this investigation were summarized for statistical analysis with descriptive statistics presented as mean ± standard deviation at all evaluations. For each treatment group, *t*-test analysis compared baseline results with corresponding post-treatment outcomes. An analysis of variance (ANOVA) compared baseline results between treatment groups. Statistical differences between treatments were determined by an analysis of covariance (ANCOVA) that included the corresponding baseline as the covariable with treatment differences evaluated by a Tukey-HSD. For all tests, statistically significant results are reported at p values less than 0.05.

Results

Subject demographics

Subjects screened for this study are shown in a CONSORT diagram (Fig. 1) with 245 evaluated for study enrollment. Two hundred twelve subjects who met the study criteria were enrolled, completed the washout phase and randomly placed in the treatment groups. Ninety-four subjects in the control group and 97 in the test group completed the entire study. Twelve subjects in the control group and 9 subjects in the test group discontinued study participation at various follow-up visits. A primary reason for subjects discontinuing study participation was relocation from the area. Other reasons for study discontinuation included non-compliance with study procedures and represented factors unrelated to the trial. The study population comprised 41 males and 150 female subjects with average age of 32.4 and 32.3 years, respectively (Table 1). The average age of subjects in the control and test groups was 32.6 and 32.3 years respectively with no significant differences by ANOVA ($p > 0.05$). Age range of subjects recorded in the control and test groups were 18–60 and 18–59 respectively with chi-square analysis demonstrating no significant differences between treatment groups for subjects' demographics ($p > 0.05$).

The effect of brushing with assigned toothpaste on dental plaque and gingival index scores is presented in Table 2 as average ± standard deviation over the study period. At baseline, analyses indicate no statistically significant differences for either dental plaque or gingival index scores ($p > 0.10$). On the other hand, treatment groups reported progressive reductions in clinical scores over the study period. Gingival index scores for the test group consistently demonstrated reductions from baseline to each post-treatment evaluation ($p < 0.05$). In contrast, the control group demonstrated no statistically significant reduction in gingival index scores from baseline to any of the post-treatment evaluations ($p > 0.05$) (Table 2).

Statistical analyses evaluating the effects of the two dentifrice treatment groups for inter-group comparisons of clinical scores over the study period are in Table 2 and reported as adjusted means at each of these evaluations. At the 4-week post-treatment evaluation, the test group demonstrated lower dental plaque and gingival index scores Table 2 than the control group that was significantly different ($p < 0.05$). Average scores for gingival and dental plaque indices were 1.21 and 2.92 respectively for the test group at the 4-week examination with the control group registering average scores for gingival index and dental plaque scores of 1.31 and 2.99, respectively. Analysis of the 4-week results indicates significantly lower

Table 1 Baseline clinical parameters of subjects enrolled in the study

Parameter	Treatment group	Baseline (Mean ± SD)	4-week (Mean ± SD)	6-week (Mean ± SD)	12-week (Mean ± SD)
Gingivitis	Test group	1.30 ± 0.24	1.20 ± 0.24	1.18 ± 0.23	0.85 ± 0.27
	Control group	1.33 ± 0.27	1.32 ± 0.27	1.32 ± 0.26	1.30 ± 0.24
Dental plaque	Test group	3.17 ± 0.36	2.92 ± 0.36	2.68 ± 0.30	2.40 ± 0.27
	Control group	3.18 ± 0.36	2.99 ± 0.34	2.75 ± 0.32	2. 64 ± 0.29
Polymorphonuclear leukocytes (PMN)	Test group	5.67 ± 0.25	5.61 ± 0.25	5.55 ± 0.25	5.40 ± 0.27
	Control group	5.65 ± 0.24	5.67 ± 0.23	5.67 ± 0.25	5.64 ± 0.22

scores for the test group in comparison to the control group ($p < 0.05$). At the 6-week examination, the test group demonstrated average scores of 1.18 and 2.68 for gingival index and dental plaque indices respectively and was significantly lower than the corresponding scores of 1.31 and 2.75 for the control group ($p < 0.05$). At the 12-week evaluation, the test group demonstrated an average gingival index score of 0.86 while the control group registered a score of 1.29 representing a 33.3% reduction that was statistically significant ($p < 0.05$). The dental plaque index demonstrated a 9.1% reduction for the test group in comparison to the control with these outcomes representing statistically significant results ($p < 0.05$).

While the test group demonstrated reductions in gingival index scores from baseline to each of the post-treatment visits ($p < 0.05$), the control group demonstrated no statistically significant reduction in gingival index scores from baseline to any of the post-treatment evaluations ($p > 0.05$).

The effect of brushing with the control and test toothpaste on oral PMN is shown in Table 3. Results are presented as average ± SD for recovered PMN (Log_{10}CFU/ml). At the baseline examination, the test and control groups registered oral PMN scores of 5.67 and 5.65, respectively. Analysis indicates no statistically significant differences between the treatment groups at baseline for oral PMN ($p > 0.05$). Brushing with the test toothpaste demonstrated progressive reductions in PMN at the 4-week, 6-week and 12-week evaluations. In contrast, brushing with the control dentifrice resulted in slight changes in oral PMN over the study duration.

Comparisons between treatment groups for effects on oral PMN are shown in Table3 with analyses conducted by ANCOVA. At the 4-week evaluation, the test group registered a significantly lower oral PMN score of 5.60 in comparison to 5.68 recorded for the control ($p < 0.05$). Analyses indicate the brushing with the test group resulted in a 16.8% lower oral PMN score than the control toothpaste at the 4-week examination. PMN scores at the 6-week evaluation were 5.54 for the test and 5.63 for the control groups respectively representing statistically significant differences ($p < 0.05$). Brushing with the test group resulted in a 18.7% reduction for oral PMN in comparison to the control. At the 12-week post-brushing evaluation, the average scores for the test and control toothpastes were 5.40 and 5.64 respectively for oral PMN scores and were significantly different ($p < 0.05$). PMN in the test group were 42.5% lower than in the control.

Discussion

Changes in oral microbial ecology and dental plaque levels induce gingival inflammation with gingivitis and periodontitis representing conditions prevalent worldwide [4, 21]. Evaluating the status of the entire mouth provides a comprehensive measure of health rather than assessments restricted to certain oral sites. Indeed we utilized this approach previously (Prasad et al., 2018) to examine effects of toothbrushing on organisms of distinct oral microenvironments including those that are not subjected to oral hygiene such as the surfaces of the cheek, tongue, gingiva and in saliva. Notably, the zinc toothpaste demonstrates reductions in viable organisms representing those capable of growth and colonization of distinct oral sites which may induce inflammatory responses. These effects were noted at evaluations conducted 12 h after brushing in comparison to a fluoride toothpaste (Prasad et al., 2018) with these results supporting outcomes from longer term evaluations reporting reductions in dental plaque and gingivitis [10]. A whole mouth antimicrobial effect representing a reduction in microbial burden prompted an assessment of oral PMN that respond to inflammatory stimuli for an assessment of whole mouth inflammation.

This double-blind clinical study compared the 12-h effects of brushing with a fluoride toothpaste to this zinc dentifrice on oral PMN over a 12-week period. PMN were enumerated after 4-week, 6-week and 12-week of toothpaste with these assessments conducted in conjunction with determinations of clinical efficacy measured as gingival inflammation and dental plaque using commonly utilized clinical indices. Several aspects were standardized for this study to enroll individuals from the general population who were not seeking any medical or dental care. Male and female subjects with gingivitis and a complement of natural teeth comprised the evaluated population with a broad age range (>18 years) to provide generalizable outcomes relevant for preventative approaches.

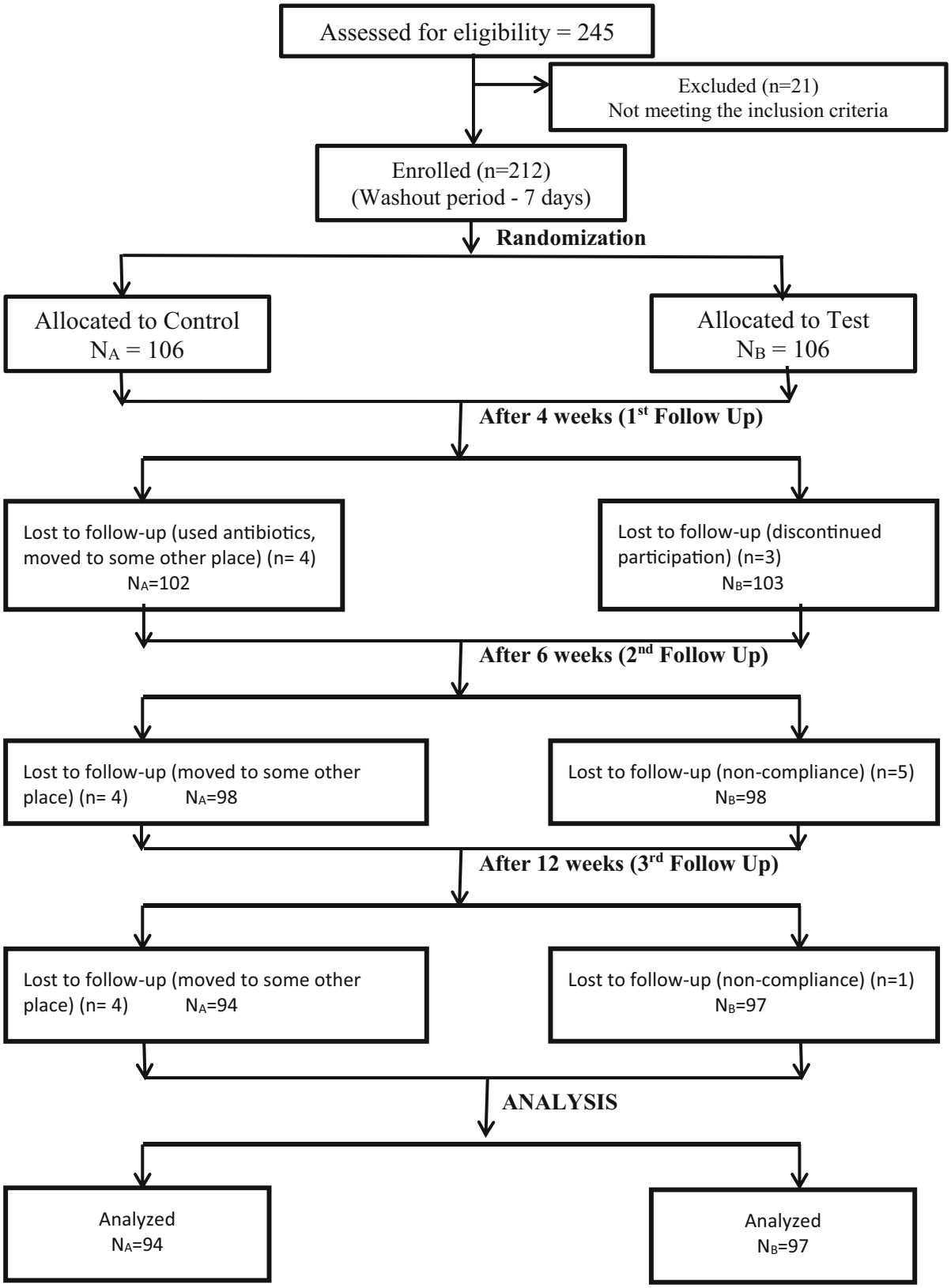

Fig. 1 Consort flow diagram showing the distribution of the study subjects through each stage of the trial

Enrolled subjects completed a washout phase, reported no previous experiences with clinical studies and registered average gingival index and dental plaque index scores of ≈1.3 and 3.1 respectively at their baseline visit representing

Table 2 Inter-group comparisons between treatments for dental plaque and gingivitis at 4-, 6- and 12-week follow-up

Time interval	Parameter	Treatment	Adj.4-week mean (S.E)	Within-treatment analysis[¶]		Between-test vs. control group[¶]	
				Reduction	Sig	Difference	Sig
4-week	Gingivitis	Test	1.21 (0.02)	7.7%	$p < 0.001$	7.6%	$p < 0.001$
		Control	1.31 (0.02)	0.8%	$p = 0.781$		
	Dental plaque	Test	2.92 (0.02)	7.9%	$p < 0.001$	2.3%	$p = 0.044$
		Control	2.99 (0.02)	6.0%	$p < 0.001$		
6-week	Gingivitis	Test	1.18 (0.02)	9.2%	$p < 0.001$	9.9%	$p < 0.001$
		Control	1.31 (0.02)	0.8%	$p = 0.781$		
	Dental plaque	Test	2.68 (0.02)	15.5%	$p < 0.001$	2.5%	$p = 0.019$
		Control	2.75 (0.02)	13.5%	$p < 0.001$		
12-week	Gingivitis	Test	0.86 (0.02)	34.6%	$p < 0.001$	33.3%	$p < 0.001$
		Control	1.29 (0.02)	2.3%	$p = 0.097$		
	Dental plaque	Test	2.40 (0.02)	24.3%	$p < 0.001$	9.1%	$p < 0.001$
		Control	2.64 (0.02)	17.0%	$p < 0.001$		

[¶] Calculated using least square means

the clinical status of the local population reported previously [40]. For this investigation, subjects were randomly provided their randomized treatment and were not instructed to change their diet or brushing habits to reduce the influences of these changes on dental plaque and oral organisms [14, 36, 37]. Included for evaluations were accepted indices for clinical activity in conjunction with oral PMN representing areas that have not been previously investigated.

PMN are described broadly as critical effector cells and identified as initial first responders [6, 27] that are produced in large numbers daily with human adults reportedly producing 100 billion neutrophils per day [3] with their production a central activity of the bone marrow [6]. Many methods for PMN enumeration such as samples stained for analysis by microscopy or flow cytometers [23] to resolve the cell types in oral washings and other types of samples have been described extensively [26]. While reductions in PMN infiltrates are associated with improving oral hygiene [18], histological analyses of gingival inflammation or of the implant mucosa [47] indicate a predominance of PMN as the principal class of leukocytes in the oral cavity. Neutrophil infiltration of gingival regions is widely recognized with increases from 2.5×10^7 PMN/ml in the connective tissue, and 1.7×10^8 PMN/ml in the junctional epithelium in minimally inflamed gingivia, to substantially higher amounts with progressing inflammation [13] with up to 30,000 cell/min migrating through the tissue [33]. Reports with an oral rinse outline approaches to collect and enumerate oral PMN [1, 5, 31, 45]. Results from the literature indicate lower oral neutrophils amongst adult subjects in good gingival health in comparison to those with periodontal disease with no differences including functional aspects of isolated oral PMN reported between genders [31]. While procedures for neutrophil assessment in this study had similarity to those reported previously [1, 5, 31, 45], an assessment of oral hygiene due to an intervention and inclusion of a distinct group of subjects with gingivitis represented evaluations that went further than available research.

At baseline, clinical parameters showed no significant differences between treatment groups but demonstrated progressive changes over the study period with the effects of the zinc

Table 3 Inter-group comparisons between treatment groups for polymorphonuclear leukocytes (PMN) $\log_{10}$(counts/ml)

Time interval	Treatment group	Adj.4-week mean (S.E)	Within-treatment analysis[¶]		Between-test vs. control group[¶]	
			Reduction	Sig	Difference	Sig
4-week	Test	1.21 (0.02)	5.60 (0.01)	12.9%	$p < 0.001$	16.8%
	Control	1.31 (0.02)	5.68 (0.01)	− 4.7%	$p = 0.037$	
6-week	Test	1.18 (0.02)	5.54 (0.01)	24.1%	$p < 0.001$	18.7%
	Control	1.31 (0.02)	5.63 (0.01)	4.5%	$p = 0.012$	
12-week	Test	0.86 (0.02)	5.40 (0.01)	46.3%	$p < 0.001$	42.5%
	Control	1.29 (0.02)	5.64 (0.01)	2.3%	$p = 0.064$	

[¶] Calculated using least square means

toothpaste markedly better than the control. Notably, the control dentifrice demonstrated no significant reductions from baseline for gingival index scores at all post-treatment evaluations and registered effects between 0.8 and 2.3%. In contrast, progressive gingival index reductions were noted amongst those brushing with the zinc toothpaste that ranged between 7.7 and 34.6% with statistically significant reductions at all evaluations. Dental plaque scores for the control group registered reductions between 6.0 and 17.0% over the study period representing significant reductions but were significantly lower than those for the zinc toothpaste that were between 7.9 and 24.3%.

The effects of toothpastes on numbers of oral PMN broadly reflected observations from the clinical indices. While the control dentifrice demonstrated a slight increase of 4.7% in PMN from baseline to the 4-week post-brushing evaluation, no significant reductions in PMN were noted for the remaining evaluations. In comparison, the zinc toothpaste registered reductions of 12.9%, 24.1% and 46.3% at the 4-week, 6-week and 12-week examinations respectively that were significantly lower than baseline. In comparison to the control, the zinc toothpaste provided PMN reductions between 16.8 and 42.5% over the study period and consistently higher than corresponding effects on the gingival index.

Zinc is the second most abundant dietary trace element in the body with influences on numerous functions [46] and a long history of use in dentistry for oral care applications. In laboratory studies, sodium lauryl sulphate (SLS) improves the solubility of zinc salts [15] with this combination demonstrating greater inhibition of supragingival plaque after clinical application. Zinc demonstrates bacteriostatic properties and readily binds bacteria including those growing as biofilms [7]. A number of microbial biochemical pathways were inhibited by zinc (Phan et al., 2004) with reductions in inflammatory end-products of *P. gingivalis* and *F. nucleatum* [35]. Whereas the effects of zinc on oral mucosa have fewer representations in the literature, tissue bound zinc reportedly modulates inflammation mediated by *H. pylori* [34]. The effects of a zinc toothpaste on bacteria found in distinct oral microenvironments [35] indicate delivery and retention features of active ingredients in the distinct regions of the human mouth.

Monitoring inflammatory activity is a relevant endpoint from the standpoint of clinical trials. Currently accepted approaches for monitoring the effects of oral hygiene interventions are based on clinical examinations that can include radiographic evidence [17, 25]. Whereas these approaches are subjective with semi-quantitative outcomes and other identified limitations [12, 17], they find extensive application and form a primary basis for investigations [12, 17]. An aspect of major strength in the present study is the longitudinal design with a series of multiple follow-ups over an extended period and inclusion of a control group. Since healing and resolution of inflammation are patient-relevant outcomes, PMN enumeration may represent an inflammatory continuum and a measure of ongoing clinical activity in contrast to classical clinical indices that are also likely inappropriate for evaluating incipient disease. PMN-relevant biomarkers such as lactoferrin and PMN elastase have been investigated with some finding acceptance for gastrointestinal conditions with FDA cleared tests and available for clinical use in the USA and Europe [20].

Improvements in oral hygiene with reductions of dental plaque and the viable organisms of distinct microenvironments capable of multiplication and translocating between oral niches (Prasad et al., 2018) highlight a decrease in the oral inflammatory burden. These observations may contribute to other outcomes based on the relationships between oral and systemic diseases. An elevated systemic neutrophil response was reported in some populations with differences noted in subjects based on race and gender during a 3-week experimental gingivitis model that restricted oral hygiene to accumulate bacterial dental plaque [19, 43] with these effects attributed to endotoxemia observed in about 56% of the evaluated subjects. In long-term epidemiology studies, higher peripheral blood neutrophil counts were reported as predictive of subsequent coronary disease amongst the healthy or those previously diagnosed with coronary disease [24, 30, 41] or diabetics [32].

This study enrolled community-dwelling adults with clinical gingivitis and excluded those who did not present with qualifying criteria or requiring dental care. Study inclusion criteria were chosen to reduce the influence of clinical variables with a sizeable number of subjects enrolled to identify effects on a broad population [31]. Results from this investigation suggest that the selected evaluations have a high likelihood for implementation in future studies to examine the effects of other oral hygiene measures, i.e. flossing, mouthrinses and other hygiene aids. Efforts indicate that these methods are suitable for surveys to examine subjects stratified by clinical status and research evaluating oral hygiene improvements by treatments including mouthwashes or dental prophylaxis (unpublished data). Notable aspects include the relatively inexpensive, safe and non-invasive approach that utilize commonly available equipment for easy interpretation of outcome measures. Furthermore, replicate analyses of collected samples can improve measurement accuracy. The study did not enroll subjects with other types of common oral conditions and enrolled subjects based on self-reporting for systemic diseases for generalizable outcomes. Future efforts can be designed to determine the effects of interventions on selected populations. Patients with gingivitis reflective of those in the population brushing with the assigned toothpaste registered a measure of reversal of gingivitis and healing that was differentiated between the treatment groups.

In summary, the results from this double-blind clinical study demonstrate that after 4 weeks, 6 weeks and 12 weeks

of subjects' twice-daily brushing with a zinc toothpaste with samples taken 12 h after oral hygiene had significantly lower numbers of PMN representing reductions in whole mouth inflammation as compared to those who brushed twice daily with a fluoride toothpaste. Additionally, at each post-treatment evaluation, subjects brushing with the zinc toothpaste demonstrated significant improvements in oral hygiene with greater reductions in gingivitis and dental plaque as compared and those provided the fluoride toothpaste. Taken together, the present study design demonstrating reductions in whole mouth inflammation complements microbiological investigations demonstrating reductions in whole mouth microbial burden by evaluating bacteria at distinct oral microenvironments (Prasad et al., 2018).

Funding This study was funded by Colgate Palmolive, USA.

Declarations

Ethical approval The study was approved by the Institutional Ethics Committee of the SDM College of Dental Sciences and Hospital and conducted in accordance with widely accepted practices for clinical studies.

Conflict of interest The authors declare no competing interests.

Informed consent Written informed consent was obtained from all the participants included in the study.

References

1. Akpek G, Knight RD, Wright DG (2003) Use of oral mucosal neutrophil counts to detect the onset and resolution of profound neutropenia following high-dose myelosuppressive chemotherapy. Am J Hematol 72(1):13–19
2. Allaker RP, Douglas CW (2009) Novel anti-microbial therapies for dental plaque-related diseases. Int J Antimicrob Agents 33(1):8–13
3. Allen RC, Stephens JT Jr (2011) Myeloperoxidase selectively binds and selectively kills microbes. Infect Immun 79(1):474–485
4. Axelsson P, Albandar JM, Rams TE (2002) Prevention and control of periodontal diseases in developing and industrialized nations. Periodontol 29:235–246
5. Bender JS, Thang H, Glogauer M (2006) Novel rinse assay for the quantification of oral neutrophils and the monitoring of chronic periodontal disease. J Periodontal Res 41(3):214–220
6. Borregaard N (2010) Neutrophils, from marrow to microbes. Immunity. 33:657–670
7. Catuogno C, Jones MN (2003) The antibacterial properties of solid supported liposomes on Streptococcus oralis biofilms. Int J Pharm 257(1-2):125–140
8. Claydon NC (2008) Current concepts in toothbrushing and interdental cleaning. Periodontol 48:10–22
9. Cummins D, Marsh PD (2018) Changing the paradigm of daily prevention to achieve whole mouth health in the 21st century. J Clin Dent. 29:A1-9
10. Delgado E, Garcia-Godoy F, Montero-Aguilar M, Mateo LR, Ryan MJ (2018) A clinical investigation of a dual zinc plus arginine dentifrice in reducing established dental plaque and gingivitis over a six-month period of product use. Clin Dent 29(Spec No A):A33–A40
11. Delima AJ, Van Dyke TE (2003) Origin and function of the cellular components in gingival crevice fluid. Periodontol 31:55–76
12. Eaton KA, Rimini FM, Zak E, Brookman DJ, Newman HN (1997) The achievement and maintenance of inter-examiner consistency in the assessment of plaque and gingivitis during a multicentre study based in general dental practices. J Clin Periodontol 24(3):183–188
13. Galicia JC, Benakanakere MR, Stathopoulou PG, Kinane DF (2009) Neutrophils rescue gingival epithelial cells from bacterial-induced apoptosis. J Leukoc Biol 86(1):181–186
14. Ganss C, Schlueter N, Preiss S, Klimek J (2009) Tooth brushing habits in uninstructed adults–frequency, technique, duration and force. Clin Oral Investig 13(2):203–208
15. Giertsen E, Scheie AA, Rölla G (1989) Plaque inhibition by a combination of zinc citrate and sodium lauryl sulfate. Caries Res 23(4):278–283
16. Haraszthy VI, Zambon JJ, Sreenivasan PK, Zambon MM, Gerber D, Rego R, Parker C (2007) Identification of oral bacterial species associated with halitosis. J Am Dent Assoc 138(8):1113–1120
17. Hefti AF, Preshaw PM (2012) Examiner alignment and assessment in clinical periodontal research. Periodontol 59(1):41–60
18. Kornman KS, Page RC, Tonetti MS (1997) The host response to the microbial challenge in periodontitis: assembling the players. Periodontol 14:33–53
19. Kowolik MJ, Dowsett SA, Rodriguez J, De La Rosa R, Eckert GJ (2001) Systemic neutrophil response resulting from dental plaque accumulation. J Periodontol 72:146–151
20. Langhorst J, Boone J, Lauche R, Rueffer A, Dobos G (2016) Faecal lactoferrin, calprotectin, PMN-elastase, CRP, and white blood cell count as indicators for mucosal healing and clinical course of disease in patients with mild to moderate ulcerative colitis: post hoc analysis of a prospective clinical trial. J Crohns Colitis 10(7):786–794
21. Löe H (2000) Oral hygiene in the prevention of caries and periodontal disease. Int Dent J 50(3):129–139
22. Löe H, Silness J (1963) Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 21:533–551
23. Loesche WJ, Robinson JP, Flynn M, Hudson JL, Duque RE (1988) Reduced oxidative function in gingival crevicular neutrophils in periodontal disease. Infect Immun 56:156–160
24. Madjid M, Awan I, Willerson JT, Casscells SW (2004) Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 44(10):1945–1956
25. Marks RG, Magnusson I, Taylor M, Clouser B, Maruniak J, Clark WB (1993) Evaluation of reliability and reproducibility of dental indices. J Clin Periodontol 20(1):54–58
26. Nakashima K, Demeurisse C, Cimasoni G (1994) The recovery efficiency of various materials for sampling enzymes and polymorphonuclear leukocytes from gingival crevices. J Clin Periodontol 21:479–483
27. Nauseef WM (2014) Myeloperoxidase in human neutrophil host defence. Cell Microbiol 16:1146–1455
28. Nibali L, Donos N, Henderson B (2009) Periodontal infectogenomics. J Med Microbiol 58(Pt 10):1269–1274
29. Offenbacher S, Barros SP, Beck JD (2008) Rethinking periodontal inflammation. J Periodontol 79(8 Suppl):1577–1584
30. Prentice RL, Szatrowski TP, Fujikura T, Kato H, Mason MW, Hamilton HH (1982) Leukocyte counts and coronary heart disease in a Japanese cohort. Am J Epidemiol 116:496–509
31. Rijkschroeff P, Jansen ID, van der Weijden FA, Keijser BJ, Loos BG, Nicu EA (2016) Oral polymorphonuclear neutrophil characteristics in relation to oral health: a cross-sectional, observational clinical study. Int J Oral Sci 8:191–198

32. Saito I, Folsom AR, Brancati FL, Duncan BB, Chambless LE, McGovern PG (2000) Nontraditional risk factors for coronary heart disease incidence among persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Ann Intern Med 133(2):81–91
33. Schiött CR, Löe H (1969) The origin and variation in the number of leukocytes in the human saliva. J Periodontal Res Suppl 4:24–26
34. Sempértegui F, Díaz M, Mejía R, Rodríguez-Mora OG, Rentería E, Guarderas C, Estrella B, Recalde R, Hamer DH, Reeves PG (2007) Low concentrations of zinc in gastric mucosa are associated with increased severity of Helicobacter pylori-induced inflammation. Helicobacter. 12(1):43–48
35. Sheng J, Nguyen PT, Marquis RE (2005) Multi-target antimicrobial actions of zinc against oral anaerobes. Arch Oral Biol 50(8):747–757
36. Signoretto C, Burlacchini G, Bianchi F, Cavalleri G, Canepari P (2006) Differences in microbiological composition of saliva and dental plaque in subjects with different drinking habits. New Microbiol 29(4):293–302
37. Signoretto C, Bianchi F, Burlacchini G, Sivieri F, Spratt D, Canepari P (2009) Drinking habits are associated with changes in the dental plaque microbial community. J Clin Microbiol
38. Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontol 38:135–187
39. Sreenivasan PK (2018) The effects of two new Dual Zinc plus Arginine dentifrices in reducing oral bacteria in multiple locations in the mouth: 12-hour whole mouth antibacterial protection for whole mouth health. J Clin Dent. 25
40. Sreenivasan PK, Prasad KVV, Javali SB (2016) Oral health practices and prevalence of dental plaque and gingivitis among Indian adults. Clin Exp Dent Res 2(1):6–17
41. Sweetman PM, Thomas HF, Yarnell JWG, Baker IA, Elwood PC (1997) Total and differential leukocyte counts as predictors of ischemic heart disease: the Caerphilly and Speedwell studies. Am J Epidemiol 145:416–421
42. Turesky S, Gilmore ND, Glickman I (1970) Reduced plaque formation by the chloromethyl analogue of victamine C. Journal of periodontology. 41(1):41–3
43. Wahaidi VY, Dowsett SA, Eckert GJ, Kowolik MJ (2009) Neutrophil response to dental plaque by gender and race. J Dental Res 88:709–714
44. Westfelt E (1996) Rationale of mechanical plaque control. J Clin Periodontol 23(3 Pt 2):263
45. Wright DG, Meierovics AI, Foxley JM (1986) Assessing the delivery of neutrophils to tissues in neutropenia. Blood. 67(4):1023–1030
46. Zalewski PD, Truong-Tran AQ, Grosser D, Jayaram L, Murgia C, Ruffin RE (2005) Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets. A review. Pharmacol Ther 105(2):127–149
47. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J (2001) Experimental peri-implant mucositis in man. J Clin Periodontol 28:517–523