A randomized trial evaluating the efficacy of ECO Balance on gingival inflammation, oral malodor and teeth whitening

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ABSTRACT

Background: A randomized clinical trial was performed to test the efficacy of a cetylpridium chloride-based post foaming gel on gingivitis, oral malodor and tooth whitening following a 6-week treatment and a 2-week post-treatment rebound. **Methods**: A total of 39 participants were included in the study and were randomly assigned to either one of two treatment groups (Group 1: formulation + device; Group 2: formulation + toothbrush) or a split-mouth control group (Group 3a: brushing; Group 3b: brushing & flossing). Clinical measures (BOP, GI, PI & PD) were chosen to reflect gingival health, tooth whiteness and breath quality. Within-treatment statistical analysis of clinical parameters from baseline to treatment and maintenance endpoints were conducted. Microbial samples taken at baseline and follow up were analyzed by DNA-DNA hybridization techniques to determine changes in subgingival flora profile. **Results**: Bleeding on probing, gingival index, plaque index, probing depth, oral malodor and tooth shade were significantly reduced in both treatment groups at 6-weeks compared to baseline ($p<0.05$). The reductions in BOP and GI for Group 1were significantly greater than brushing & flossing ($p_{BOP}=0.007$; $p_{GI}=0.036$). Participants in Group 1 experienced sixteen-times greater reduction in bleeding and plaque than brushing & flossing. Participants using the formulation saw significant reductions of periopathogens greater than control, in addition to reduced malodor and whiter teeth. **Conclusion**: The results indicate that the foaming gel formulation significantly reduces gingivitis, freshens breath and whitens teeth.

1. Introduction

Periodontal diseases, comprising gingivitis and periodontitis, are multifactorial inflammatory infections caused by pathogenic bacteria among the tooth surface biofilm that are in part responsible for tooth-supporting tissue destruction[1]. According to the Centers for Disease Control and Prevention (CDC), periodontitis affects nearly half of American adults (47%; roughly 65 million patients over the age of thirty)[2], which suggests that current strategies to control periodontal inflammation have not been effective on a population level and renewed efforts in therapeutic innovation are necessary.

Research has indicated that the initiation and progression of periodontal disease is influenced by a small proportion of gram-negative anaerobes (i.e., *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*)[3]. These bacteria exist within the dental plaque biofilm and trigger the host inflammatory response. While the etiology of periodontal disease is bacterial, the host inflammatory response is responsible for periodontal destruction[4], as disease is not due to bacterial influence alone[5].

Consistent with a nonspecific model of microbial pathogenesis, strategies for prevention and treatment of gingivitis and mild periodontitis are dependent on the reduction of plaque mass[6] at the gingival margin. It is expected that by lowering plaque levels, a reduction in disease severity will result, however the relationship of supragingival plaque mass to the severity of gingival inflammation is not linear and varies between individuals[7]. Although mechanical removal of plaque remains the primary means of controlling periodontal inflammation, the search for safe and effective chemical agents for plaque control is still an important clinical problem[8]. The most compelling chemotherapeutic agent currently available is the antiseptic, chlorhexidine (CHX), which has demonstrated short-term efficacy in situations where mechanical plaque removal is impaired, however it is limited in long-term usage by side effects such as harsh stains and taste[9]. Further, CHX is largely compromised by its display of microbicide resistance in clinical isolates of *Staphylococcus aureus*[10], and its contribution to the development of multidrug resistance following long term use[11]. This is of major importance as the careless use of broad spectrum antiseptics may lead to the development of bacterial strains that can occupy a resistant nice in plaque biofilm[12] that can serve as a source of chronic infection[13,14].

The current standard of care by which dental professionals respond to a periodontitis diagnosis is scaling and root planing (SRP), and adjuncts to this procedure may include local or systemic antibiotics. However, both local and systemic antibiotic therapies have shown moderate to small benefit with low levels of certainty, at best[15]. In a recent systematic review[16], researchers evaluated the effects of localized antimicrobials as adjuncts to SRP in patients with periodontitis and diabetes mellitus. The researchers determined that local antimicrobials used adjunctively to SRP may improve pocket depth (PD) and clinical attachment level (CAL), however four trials[17-20] observed some difference between groups, whereas two trials[21,22] did not find an additional benefit of using minocycline. Although the researchers identified a few studies that showed clinical improvements beyond SRP alone, the PD reductions (0.4mm) and CAL gains (0.31mm)[23] were minor compared to the baseline PD. Thus, there exists a need for an alternate nonsurgical method for controlling the microbial profile and subsequent inflammatory response to pathogens, that is non-antibiotic to eliminate the potential of exacerbating

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the public health issue of antibiotic resistance.

In addition to controlling inflammation for the immediate stabilization of the periodontal condition, a growing body of research over the last decade has clearly indicated a link between periodontal disease and systemic inflammation[24-32]. The sub-speciality of medicine dedicated to the link between oral and overall health connects dentistry and medicine in a way that was previously neglected, which is of utmost importance as respective therapies have been shown to have an influence on each other's treatment outcome[27,30,32].

According to the American Academy of Cosmetic Dentistry (AACD), the annual revenue of the teeth whitening industry exceeded $11 billion (USD) in 2015[33], which translates to millions of Americans electing to whiten their teeth each year. While it is known that nearly 70% of the U.S. adult population suffers from a form of gingival disease, it can be deduced with high probability that many people are receiving cosmetic dentistry in the context of inflammation. There is a lacking volume of research to date evaluating whitening in patients with periodontal disease and thus a need exists to assess both aesthetic and clinical endpoints simultaneously.

Recently, a novel cetylpyridinium chloride-based foaming gel containing hydrogen peroxide and sodium bicarbonate was introduced as an antigingivitis treatment. The agent was introduced with whitening and breath freshening claims, however there have been no published clinical studies conducted with this gingival agent. Therefore, in the current study, the treatment of gingivitis, plaque removal and inhibition, control of inflammation, breath freshening, tooth whitening and microbial profile alteration by the interventional foaming gel with and without an accelerating device were compared with those functions of an antioxidant & whitening OTC fluoride toothpaste. The aims of the research are to assess the safety and efficacy of the foaming gel, administered with and without an accelerating device, on the reduction of gingival inflammation, bleeding on probing (BOP), pocket depth (PD), plaque, halitosis (Volatile Sulfur Compounds; VSCs), improvement of tooth color and alteration of subgingival microbial profile. It is hypothesized that participants would experience gingival clinical improvements, enhanced tooth shade, reduced VSCs and microbial presence when using the foaming gel with the device and/or on a toothbrush, greater than the control group.

2. Materials and Methods

2.1. Study design

A 60-day randomized, single blinded parallel group study was conducted on thirty-six medically healthy participants with existing gingivitis and/or mild periodontitis. Participants did not receive initial periodontal therapy and were randomized using permuted block randomization to one of three groups as follows: Group 1 (n=12) participants were instructed to brush their teeth with a standard toothbrush and antioxidant whitening toothpaste twice daily, as well as perform an 8-minute application of the interventional product with the device; Group 2 (n=12) participants were instructed to brush their teeth with a standard toothbrush and antioxidant whitening toothpaste twice daily, as well as brushing with ECO Balance on top of their toothpaste once daily; Group 3 (control; n=12) participants were instructed to brush their teeth with a standard toothbrush and antioxidant whitening twice daily, in addition to flossing half of their mouth (right side, upper and lower) once daily (split mouth design; Group 3a: flossed; Group 3b: non-flossed). All participants were evaluated at baseline and follow-up visits (days 14, 28, 42 & 60) for the following clinical parameters: Probing \Depth (PD), Bleeding on Probing (BOP), Gingival Index (GI)[34] and Plaque Index (PI)[34]. All participants were also evaluated at baseline and follow-up visits for the following biological parameters: halitosis (Volatile Sulfur Compounds; VSCs), subgingival plaque microbial profile, inflammatory cytokines/chemokines and matrix metalloproteases (MMPs), and tooth color (VITA Shade). At day 42, all test product use was discontinued, and participants returned for a final visit on day 60 to assess maintenance.

2.2. Participant selection and randomization

Participants were recruited from the subject pool at Forsyth Center for Clinical and Translational Research (CCTR). Potentially eligible participants were screened and thirty-six participants that met inclusion and exclusion criteria were enrolled. Three participants electively withdrew during the trial, and three additional participants were recruited to maintain a sample size of thirty-six (n=36). Participants recruited were in good health and exhibited the characteristics outlined in Table 1.

Table 1 – Clinical trial participant eligibility criteria.

Inclusion Criteria	Exclusion Criteria
• Willing and able to read, understand and sign an Informed Consent form. • Good general health as evidenced by the medical history. • Between 18 and 55 years of age. • Male or female • A minimum of 20 teeth, excluding crowns and third molar teeth. • A mean whole mouth GI of ≥2.0 at baseline. • Sites with ≤7 mm pocket depth. • Willing to abstain from oral hygiene procedures for 12-18 hours prior to clinical visits. • Willing to abstain from chewing gums, oral whitening products, mouthwashes and tobacco products for the study duration. • Able to understand and follow study directions.	• Chronic use of photosensitizing medications including NSAIDs, antidepressants, antibiotics and beta-blockers. • Diagnosed with diabetes. • Presence of orthodontic appliances. • Presence of large restorations, crowns or veneers at the anterior of both upper and lower teeth (including premolar teeth). • A soft or hard tissue tumor of the oral cavity. • Carious lesions requiring immediate treatment. • Severe internal (tetracycline stains) and external discoloration (fluorosis). • Diagnosis of severe chronic periodontitis, aggressive periodontitis, acute necrotizing ulcerative gingivitis or generalized gingival recession >2mm as evidenced by clinical oral exam. • Participating in another clinical trial or oral product study. • Pregnant or breast-feeding women. • Allergy to home bleaching products such as hydrogen peroxide and carbamide peroxide. • Use of antibiotics within 3 months of enrollment. • History of drug use that is associated with gum overgrowth (i.e., Dilantin, nifedipine, etc.). • Chronic use of medication such as steroids, anti-coagulant medications, immunosuppressant medications or any other medications or medical conditions that in the opinion of the investigator would interfere with the evaluation or confound interpretation of the study results. • Medical condition that requires pre-medication prior to dental visits/procedures. • Current smoker or former smoker within one year of enrollment.

Participants taking systemic antibiotics were excluded as the antibiotic treatment can influence the bacterial composition of the plaque biofilm as well as inflammatory changes in a matter that cannot be easily predicted. As this study assessed the effects of an intervention on gingival inflammation, participants who had prior medical conditions or were on medications known to affect periodontal tissues and inflammation were excluded as well as participants with severe periodontal disease. Participants with orthodontic appliances were excluded because plaque assessment and removal can be difficult with this population. Pregnancy and lactation may cause gingival tissue changes due to hormone level alteration that may confound the evaluation and were therefore listed as exclusion criteria. Current and former smokers within 1 year of enrollment were excluded due to smoke's effect on tooth color, oral malodor and gingival inflammation.

Written informed consent were obtained from all participants prior to their enrolment. The consent form complied with all applicable regulations governing protection of the participants, and include basic elements specified in the U.S. Code of Federal Regulations, 21 CFR 50.25(a) & 50.27, and ICH-GCPs, Chapter 4, subpart 4.8. Each participant was given unlimited time to read the consent form and ask questions. All informed consent forms were documented in a log by date and subject ID. Participants had the right to withdraw consent at any time. There were no adverse events reported throughout the trial duration.

2.3. Clinical measurements & procedures

A detailed study protocol was approved by the Forsyth IRB and is registered at clinicaltrials.gov (NCT03196648). Thirty-six participants were randomly assigned by the Forsyth Institute using permuted block randomization to one of three groups previously outlined (see *Study Design*). All clinical measurements were performed under the same conditions by the same investigator to avoid inter-rater variability.

Clinical periodontal measurements including MPD, BOP, GI and PI were recorded at baseline and each of the following visits: days 14, 28, 42 & 60. MPD was measured with a UNC-12 periodontal prove at six sites per tooth rounded to the next lower whole millimeter. BOP was assessed after probing, using a dichotomous scoring system (1 and 0, for presence or absence, respectively) at six sites per tooth. GI was assessed by placing the periodontal probe under the gingival margin and sweeping along the buccal and lingual surfaces, with notation of tissue quality and bleeding. PI was assessed by sweeping the periodontal probe along the buccal and lingual surfaces, with notation of plaque abundance. GI and PI characteristics are described in Table 2.

Table 2 – Gingival and plaque indices of Silness and Loe.

Gingival Index (GI)[34]		Plaque Index (PI)[34]	
0	Normal gingiva	0	No plaque
1	Mild Inflammation (Slight change in color, slight edema and no bleeding on probing)	1	Film at gingival margin
2	Moderate Inflammation (Redness, edema, glazing and bleeding on probing)	2	Moderate plaque (easily visible)
3	Severe Inflammation (Marked redness and edema, ulceration and tendency to bleed)	3	Abundance of plaque material

Assessment of aesthetic clinical endpoints including tooth color and breath measurements were conducted at baseline and each of the following visits: days 5, 14, 28, 42 & 60. Tooth color was measured on four maxillary incisors only using procedures accepted by the ADA for submissions of other whitening products. Shade value (SV) was assessed using the VITA Shade Guide, described in Table 3. Chromatograph measurements of breath samples were analyzed using the OralChroma device, which measures the volatile sulfur compounds (VSCs) hydrogen sulfide, dimethyl sulfide and methyl mercaptan (the major causative factors in halitosis) and displays each gas concentration. The VSC measurements were conducted by manufacturer product instruction.

Table 3 – Standardized shade values of the VITA Shade Guide.

Shade	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
Order	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Lightest															***Darkest***

Biological assessments including analysis of plaque microbial profile and gingival crevicular fluid (GCF) proinflammatory proteins, were evaluated at baseline and a follow-up visit at 28 days. Subgingival plaque samples were collected to assess microbial profile using sterile periodontal curettes (Gracey 11/12), from mesiobuccal surfaces of two teeth at each quadrant selected based on GI score. Plaque samples were placed into Eppendorf tubes containing 150 uL TE buffer and were frozen and stored at Forsyth Institute until study completion for DNA-DNA Hybridization Checkerboard analysis (Socransky, Smith et al., 1994). GCF samples were obtained from 4 sites, 1 per quadrant based on interproximal papilla redness. GCF flow was collected using standard filter paper strips (OraFlow, Inc.) from mesiobuccal aspects of all maxillary and mandibular premolars and molars. Prior to measurement, the Periotron instrument was standardized using 1uL of water on a filter paper strip. In collecting samples, a filter paper strip was placed in the tooth crevice for 30 seconds to obtain sufficient fluid. The strips were then placed in labeled Eppendorg tubes and immediately frozen in liquid nitrogen, before being transported to the laboratory for storage and analysis. All GCF samplings were obtained prior to clinical measurements and microbiological sampling. The GCF samples were analyzed for inflammatory cytokines/chemokines and matrix metalloproteases (MMP) including IL-1B, TNF-a, IL-6, IL-8 and MCP-1 using multiplexing ELISA (Luminex) at the Forsyth Institute.

2.4. Data collection and analysis

The number of participants was estimated based on previous studies to reach a significant difference in change of gingival index between intervention and control groups after 6 weeks of treatment. Using the normal approximation for sample size determination concerning two independent samples t-tests and assuming a 12% reduction (with respect to GI), alpha=0.05 and 80% power, a sample size of 12 per group will be required[8].

The primary clinical outcomes assessed were differences in BOP and GI from baseline to 14, 28 and 42 day endpoints. Secondary outcomes included differences in subgingival microbiota, inflammatory markers, and changes in PI, VSCs, PD and tooth shade.

Mean changes from baseline to each post baseline time points were compared using repeated measures ANOVA. All statistical analyses were conducted at $p<0.05$ level of significance. Within-treatment changes from baseline were analysed using reported measures of ANOVA and post hoc analysis. Between treatment changes were calculated for GI, PI, BOP and PD. In addition, descriptive statistics were calculated for all the above

parameters.

To detect changes in the subgingival bacterial profile, the DNA-DNA hybridization (checkerboard) analysis[35] was performed to provide a quantitative assessment of 16 oral bacteria. Counts of individual species were averaged within participants and then averaged across participants for baseline and follow up. Significance of differences over time were analysed using paired sample *t*-tests for within-treatment changes from baseline.

3. Results

Table 4 – Demographic features of the participants in treatment and control groups.

Parameter	Group 1	Group 2	Group 3	*P* value (Group 1)	*P* value (Group 2)
Age (mean ± SD)	43.3 ± 11.1	39.1 ± 11.9	39.1 ± 12.6	0.399	1.000
Gender					
Male	33.3%	50%	58.3%		
Female	66.7%	50%	41.7%	0.237	0.697
Race					
African-American	16.7%	25.0%	58.3%		
Asian	16.7%	8.3%	0%		
Caucasian	50.0%	50.0%	33.3%		
Hispanic	16.7%	0%	0%		
>One	0%	16.7%	8.3%	0.998	0.999

Table 5 – Baseline clinical parameter means (with 95% CI) and between-group differences of treatment to control groups.

Parameter	Mean	95% CI	*P* value (Group 1)	*P* value (Group 2)
Bleeding on Probing (%)				
Group 1	36.583	±5.727		
Group 2	32.833	±6.464		
Group 3a	35.333	±8.960	0.820	0.662
Group 3b	33.691	±6.279	0.534	0.892
Gingival Index				
Group 1	1.91	±0.141		
Group 2	1.81	±0.087		
Group 3a	1.87	±0.064	0.506	0.303
Group 3b	1.81	±0.093	0.191	0.943
Plaque Index				
Group 1	1.23	±0.200		
Group 2	1.37	±0.229		
Group 3a	1.17	±0.282	0.702	0.244
Group 3b	1.14	±0.307	0.598	0.200
Probing Depth (mm)				
Group 1	2.28	±0.203		
Group 2	2.11	±0.168		
Group 3a	2.33	±0.209	0.691	0.078
Group 3b	2.33	±0.211	0.950	0.079
Tooth Shade (*see Table 3 for description*)				
Group 1	8.45	±1.498		
Group 2	8.04	±1.449		
Group 3	7.82	±1.747	0.478	0.789
Volatile Sulfur Compounds (ppb)				
Group 1	61.73	--		
Group 2	48.45	--		
Group 3	37.30	--	0.152	0.369

3.1. Demographics

A total of 39 participants were recruited for the study. Of the original 39 participants, 36 participants completed the trial duration. Twelve participants were in the control group (Group 3) and twelve participants were in each of the treatment groups (Group 1 & Group 2). Trial protocol compliance was good, based on daily diary entries. Demographic information of the participants is outlined in Table 4. Participants in the control group were not significantly different from participants in either test groups with respect to age, gender and race.

3.2. Baseline clinical parameters

Baseline clinical parameters for all treatment and control groups are outlined in Table 5. The data are represented as full mouth averages, and the units of each parameter are indicated adjacently. Tooth shade are reported as numerical data transformed from the VITA Shade Guide, outlined in Table 3. There were no significant differences in baseline measures between either treatment and control groups.

3.3. Periodontal clinical endpoints

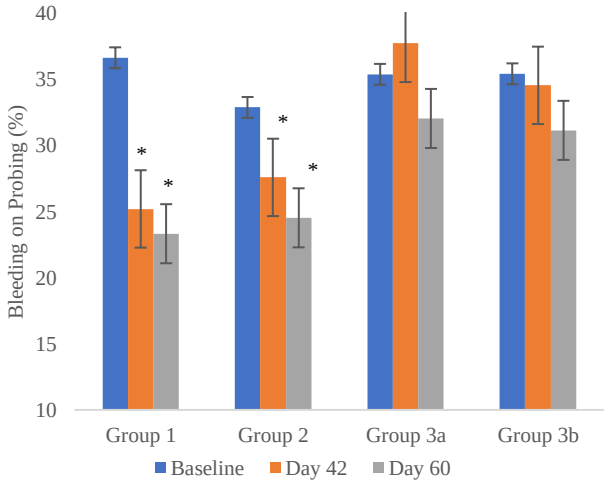

Fig. 1 – Mean BOP percent scores (with standard error) in treatment and control groups at baseline, treatment (Day 42) and maintenance (Day 60) endpoints. *Statistically significant difference from baseline.

Both treatment groups (Group 1 & 2) demonstrated statistically significant reductions in BOP from baseline to day 42 of 31.2% (p=0.00) and 16.1% (p=0.03), respectively (Figure 1). Group 3b saw a BOP reduction of 2.4% (p=0.41), whereas Group 3a resulted in increased bleeding, on average, of 6.6% (p=0.26) after 42 days, without statistical significance. Percent changes of BOP for all groups are presented in Figure 2. The between-group difference of Group 1 to Group 3a was statistically significant (p=0.00) and nearly 16 times greater at day 42.

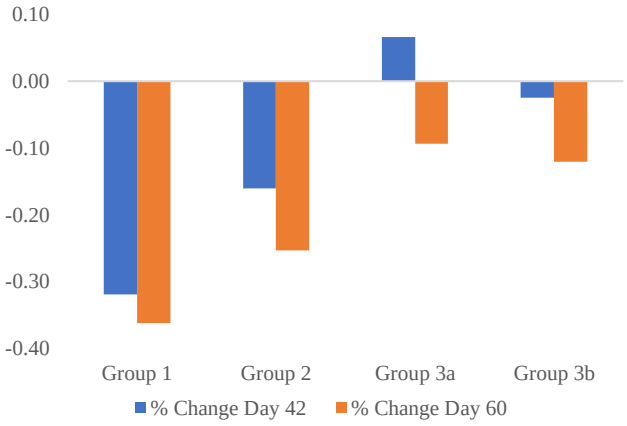

Fig. 2 – Percent change in BOP score during the treatment (day 42) and maintenance (day 60) periods. The decreases in Groups 1 & 2 are both statistically significant (p_1=0.004, p_2=0.031) compared with the change in control group (Group 3a).

Figure 3 demonstrates the mean GI scores (with SE) in all study groups at baseline, day 42 and day 60 endpoints. Groups using the interventional product experienced statistically significant within-treatment reductions in gingival inflammation (p_1=0.008; p_2=0.031), while the control group differences were minimal and insignificant (p_{3a}=0.382; p_{3b}=0.921). Group 1 between-treatment differences compared to Group 3a were statistically significant (p=0.025). Group 2 between-treatment differences compared to Group 3a and Group 3b were statistically significant (p=0.001; p=0.005, respectively). Percent changes of GI for all groups are presented in Figure 4. The percent change in Group 1 resulted in a 12% in GI decrease after 42 days, compared to a 1.6% decrease (7.5 times lesser) for Group 3a. When the interventional product was used on a toothbrush (Group 2), a 13.8% decrease in GI resulted after 42 days, whereas the control group (Group 3b) saw no change in GI score from baseline to day 42.

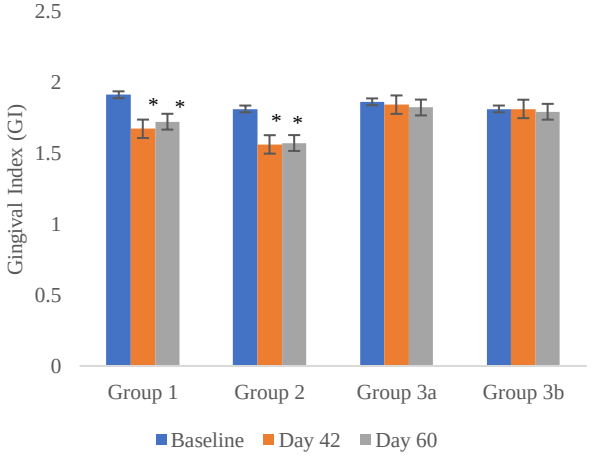

Fig. 3 –Mean GI scores at baseline, 42 and 60 day endpoints (with standard error). Within-treatment changes in baseline for Group 1 (p=0.008) and Group 2 (p=0.031) were statistically significant, unlike control groups.

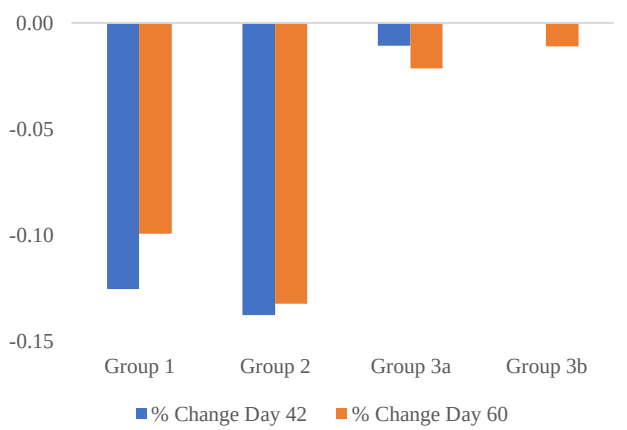

Fig. 4 –Percent change in GI scores at baseline, 42 and 60 day endpoints. The between-treatment decreases in Groups 1 & 2 are both statistically significant (p_1=0.025; p_2=0.005) compared with the change in control group (Group 3a)

Table 6 shows the baseline, treatment and maintenance clinical endpoint means, percentage changes (with 95% CIs) and within-treatment independent *t*-test significance levels for PI and full mouth PD. The interventional product used in either application resulted in significant reductions in mean PI of 29.3% (Group 1; p=0.00) and 28.5% (Group 2; p=0.01), compared to the average 6.8% of plaque accumulation observed with participants brushing with toothpaste alone (Group 3a; p=0.18) and the slight PI reduction of 1.8% observed with brushing & flossing (Group 3b; p=0.45). Participants administering the interventional product with the device (Group 1) experienced full mouth generalized PD reduction with statistical significance (p=0.05), however the differences were not clinically relevant as the baseline MPD represented healthy pocketing, generally. Full mouth MPD changes in all other groups were not found to be statistically significant (p>0.05).

Table 6 – Treatment & maintenance endpoint means, percentage change (with 95% CI) and within-treatment differences.

			Group 1	Group 2	Group 3a	Group 3b
Plaque Index (PI)	Day 0	Mean	1.23	1.37	1.17	1.14
	Day 42	Mean	0.87	0.98	1.25	1.12
		% change	-29.3%	-28.5%	6.8%	-1.8%
		p value	0.003*	0.005*	0.18	0.45
	Day 60	Mean	1.13	0.98	1.3	1.19
		% change	-8.1%	-28.5%	11.1%	4.4%
		p value	0.14	0.006*	0.06	0.30
Probing Depth (PD; mm)	Day 0	Mean	2.28	2.11	2.33	2.33
	Day 42	Mean	2.21	2.04	2.34	2.33
		% change	-3.1%	-3.3%	0.4%	0%
		p value	0.055*	0.09	0.42	0.46
	Day 60	Mean	2.23	2.02	2.3	2.26
		% change	-2.2%	-4.3%	-1.3%	-3.0%
		p value	0.1	0.02*	0.22	0.02

Table 7 shows the baseline, treatment and maintenance endpoint means, percentage changes within-treatment significance levels of average

Table 7 – Means and percentage changes of study groups localized probing depth (in mm) categorized by periodontal disease severity. *Statistically significant within-treatment differences from baseline.

			Group 1	Group 2	Group 3a	Group 3b
Healthy gingiva (1 – 3mm)	Baseline	Mean	2.122	2.020	2.152	2.125
	# of sites		n=1823	n=1894	n=891	n=881
	Day 42	Mean	2.087	1.970	2.200	2.152
		% change	-1.6%	-2.5%	2.2%	1.3%
		P value	0.401	0.360	0.379	0.591
	Day 60	Mean	2.115	1.953	2.147	2.079
		% change	-0.3%	-3.3%	-0.2%	-2.2%
		P value	0.833	0.098	0.891	0.235
Mild - Moderate Periodontitis (4 – 6mm)	Baseline	Mean	4.123	4.111	4.350	4.240
	# of sites		n=148	n=80	n=57	n=76
	Day 42	Mean	3.651	3.522	3.743	3.674
		% change	-11.4%	-14.3%	-13.9%	-13.3%
		P value	0.001*	0.027*	0.002*	0.004*
	Day 60	Mean	3.534	3.517	3.647	3.566
		% change	-14.3%	-14.4%	-16.1%	-15.9%
		P value	0.000*	0.022*	0.003*	0.002*
Severe Periodontitis (≥7mm)	Baseline	Mean	7.000	--	7.000	7.000
	# of sites		n=1	n=0	n=6	n=3
	Day 42	Mean	4.000	--	6.667	7.000
		% change	-42.9%	--	-4.8%	0%
		P value	--	--	--	--
	Day 60	Mean	4.000	--	6.667	0.667
		% change	-42.9%	--	-4.8%	-4.8%
		P value	--	--	--	--

Localized PDs, categorized by periodontal disease severity. Periodontal pocketing categorized as healthy gingiva (1-3mm) did not experienced statistically significant reductions across all groups, which is expected. When evaluating PDs of 4-6mm, there were statistically significant reductions across all groups, with the greatest reduction in Group 2 (14.3%; p=0.001). Due to the study's exclusion criteria, the participants presented with mild-moderate periodontal disease at most, therefore there was a very small sample of severe local pockets ≥7mm that did not allow for statistical analysis. However, a single participant in Group 1 whom had a 7mm site saw a 3mm reduction (43%) after 42 days.

3.4. Aesthetic clinical endpoints

Table 8 demonstrates the baseline, day 5, day 42 (end treatment) and day 60 (maintenance) endpoints of both breath and tooth color measurements for treatment and control groups. The breath measurements' significance levels reflect the within-treatment differences from baseline in each of the VSCs listed in the column [H_2S, CH_3SH, $(CH_3)_2S$, respectively]. Participants in Group 1 experienced statistically significant reductions in at least one VSC tested (H_2S and/or CH_3SH) at day 5 (p=0.00), day 42 (p=0.05; p=0.01) and day 60 (p=0.04). All other group differences from baseline did not hold statistical significance. Similarly, with regards to tooth shade, Group 1 were the only participants

Table 8 – Halitosis & teeth whitening baseline, treatment and maintenance endpoint means, difference and within-significance levels.

			Group 1	Group 2	Control
Volatile Sulfur Compounds (VSCs; ppb: H_2S, CH_3SH, $(CH_3)_2S$)	Baseline	Mean	61.73	48.45	37.3
	Day 5	Mean	40.91	74.92	38.1
		Difference	21.12	-26.47	-0.8
		p value	0.24; 0.003*; 0.25	0.37; 0.45; 0.08	0.16; 0.16; 0.13
	Day 42	Mean	20.53	60.1	47.7
		Difference	20.82	-11.65	-10.4
		p value	0.05*; 0.01*; 0.34	0.24; 0.34; 0.27	0.0; 0.11; 0.11
	Day 60	Mean	47.7	49.8	44.3
		Difference	14.03	-1.35	-7
		p value	0.04*; 0.41; 0.27	0.41; 0.33; 0.25	0.32; 0.06; 0.08
Tooth Shade (VITA Shade Guide standardized values)	Baseline	Mean	8.45	8.04	7.82
	Day 5	Mean	7.91	7.92	7.73
		Difference	0.54	0.12	0.09
		p value	0.03*	0.38	0.17
	Day 42	Mean	7.45	7.36	7.16
		Difference	1.0	0.68	0.66
		p value	0.007*	0.18	0.06
	Day 60	Mean	7.39	7.77	7.11
		Difference	1.06	0.27	0.71
		p value	0.007*	0.24	0.06

to experience statistically significant shade changes at each endpoint. After 5 days, Group 1 participants saw an average change from baseline of 0.54 shades (p=0.03), 1.0 shades after 42 days (p=0.00) and 1.06 shade after 60 days (p=0.00). Groups 2 and 3 experienced nearly two-thirds of a shade change from baseline to treatment end, that did not reach statistical significance (p=0.18 & p=0.06, respectively). All endpoint mean differences from baseline in Groups 2 and 3 were not found to be significant.

3.5. Biological endpoints

Bacterial counts of several species were statistically analysed using paired sample *t*-tests to determine within-treatment differences from baseline to 28 day follow up (Table 9). Table 9 demonstrates the bacterial load changes from baseline of sixteen subgingival taxa categorized by Socransky and colleague[36]. Socransky identified several bacteria that are directly related to clinical measures of periodontal disease (red complex), such as PD and BOP, including: *P. gingivalis*, *A. actinomycetemcomitans*, *Treponema denticola* and *Bacteriodes forsythia*. Participants in either treatment group experienced statistically significant reductions in the counts of two of the above periodontal pathogenic species (Group 1: *P. gingivalis* p=0.036, *A. actionmycetemcomitans* p=0.023; Group 2: *P. gingivalis* p=0.036. *T. denticola* p=0.042). Group 2 also experienced statistically significant reductions in two other subgingival taxa categorized under the "orange complex" (*Campylobacter rectus*: p=0.014; *Fusobacterium periodonticum*: p=0.033). Socransky found it apparent that with increasing colonization of these orange complex species, more sites were colonized with increased numbers of red complex species[36]. The control groups not using the interventional product (Groups 3a & 3b) did not see a statistically significant reduction in any of the subgingival taxa analysed. Further, the control group that did not floss (Group 3a) saw a statistically significant increase in bacterial count of the species *Streptococcus gordonii* (78% increase; p=0.016). Of the subgingival taxa analysed, participants in Group 1 saw a clinical reduction in at least 75% of species, on average. Group 2 had clinical reductions in at least 93% of species, on average, whereas Groups 3a & 3b reduced 31% and 100% of species, respectively, however without statistical significance.

Table 9 – Baseline and follow-up bacterial counts of all study groups following treatment. *Statistically significant reduction from baseline. †Statistically significant increase from baseline.

Bacterium		Bacterial CFU Count (10^5)											
		Group 1			Group 2			Group 3a			Group 3b		
		Baseline	Day 28	*P* value	Baseline	Day 28	*P* value	Baseline	Day 28	*P* value	Baseline	Day 28	*P* value
Red Complex	*P. gingivalis*	0.381	0.209	0.036*	1.016	0.318	0.036*	3.572	3.773	0.242	8.442	2.725	0.165
	T. denticola	0.763	0.477	0.109	0.988	0.351	0.042*	1.632	2.477	0.085	18.608	4.925	0.153
	T. forsythia	1.338	0.909	0.106	5.772	0.619	0.076	6.181	4.298	0.270	19.367	3.008	0.135
	A. action.	0.060	0.028	0.023*	0.050	0.027	0.103	0.042	0.033	0.160	0.075	0.025	0.070
Orange Complex	*P. intermedia*	1.063	0.824	0.303	1.467	1.488	0.488	0.999	3.535	0.075	28.450	9.600	0.182
	F. nucleatum	1.947	1.193	0.182	3.127	1.144	0.471	2.121	1.487	0.193	4.800	4.158	0.379
	P. micra	0.525	0.617	0.291	0.600	0.573	0.411	0.636	0.814	0.189	1.550	1.208	0.712
	C. rectus	0.557	0.353	0.118	0.608	0.242	0.014*	0.480	0.465	0.445	1.175	0.833	0.202
	F. periodont.	1.156	0.938	0.330	1.287	0.726	0.033*	0.979	0.950	0.459	2.542	1.867	0.289
	E. nodatum	0.710	0.736	0.441	0.843	0.494	0.021	0.700	1.665	0.165	2.850	1.967	0.112
Yellow Complex	*S. sanguis*	0.194	0.228	0.322	0.197	0.158	0.328	0.137	0.243	0.171	0.283	0.158	0.051
	S. gordonii	0.129	0.186	0.212	0.125	0.092	0.251	0.067	0.119	0.016†	0.142	0.092	0.070
	S. intermedia	0.281	0.206	0.164	0.265	0.166	0.179	0.206	0.287	0.193	0.350	0.325	0.421
Green Complex	*C. gingivalis*	1.167	1.090	0.416	0.821	0.407	0.070	0.643	0.693	0.378	1.950	0.833	0.154
	C. ochracea	1.272	0.533	0.109	1.130	0.328	0.073	0.460	0.815	0.201	2.217	1.450	0.291
	E. corrodens	1.152	0.903	0.294	1.703	1.138	0.324	0.944	1.242	0.325	2.425	0.958	0.136

Proinflammatory cytokines & chemokines of all study groups' GCF samples were statistically analysed using paired sample *t*-tests to determine within-treatment differences from baseline to day 28 follow up (Table 10). Table 10 demonstrates the means, significance and percent changes from baseline to endpoint in cytokine & chemokine volume. Groups 1 and 2 means represent the average signalling protein volume from two pockets in opposite quadrants (1 and 3) for each participant, whereas Groups 3 and 3b means represent the average signalling protein volume of one pocket in a particular quadrant depending on the split-mouth design (1 or 3) for each participant. Group 1 presented statistically significant decreases in four of the five cytokines/chemokines analysed (IL-1b, IL-6, IL-8 and TNF-a) of 76% (p=0.039), 86% (p=0.019), 84% (p=0.014) and 93% (p=0.018), respectively. Group 2 also presented statistically significant decreases in four of the five cytokines/chemokines analysed (IL-6, IL-8, MCP-1, TNF-a) of 89% (p=0.009), 83% (p=0.009), 73% (p=0.020) and 92% (p=0.014), respectively. Group 3a demonstrated a similar trend in signalling protein reduction as Group 2, in that four of five cytokines/chemokines analysed decreased with statistical significance [IL-6 (p=0.002); IL-8 (p=0.003); MCP-1 (p=0.007); TNF-a (p=0.030)]. Group 3b saw the least amount of average proinflammatory protein reduction of all groups, in that two of five cytokines/chemokines analysed had a significant change from baseline [IL-1b (p=0.024); IL-8 (p=0.009)].

Table 10 – Baseline and endpoint proinflammatory cytokines & chemokines means and percent changes of all study groups following treatment. *Statistically significant reduction (p<0.05) from baseline.

			Group 1	Group 2	Group 3a	Group 3b
IL-1β (pg/ml)	Baseline	Mean	211.10	326.31	559.12	447.51
	Day 28	Mean	49.82	35.04	59.29	54.12
		% change	-76%	-89%	-89%	-88%
		P value	0.039*	0.092	0.060	0.024*
IL-6 (pg/ml)	Baseline	Mean	12.22	17.31	90.45	93.03
	Day 28	Mean	1.72	1.98	4.44	5.55
		% change	-86%	-89%	-95%	-90%
		P value	0.019*	0.009*	0.002*	0.099
IL-8 (pg/ml)	Baseline	Mean	6676.76	4771.83	8044.23	7747.73
	Day 28	Mean	1042.56	791.70	1336.35	1631.89
		% change	-84%	-83%	-83%	-79%
		P value	0.014*	0.009*	0.003*	0.009*
MCP-1 (pg/ml)	Baseline	Mean	63.80	78.88	240.42	160.80
	Day 28	Mean	32.35	21.26	41.60	52.02
		% change	-50%	-73%	-83%	-68%
		P value	0.056	0.020*	0.007*	0.053
TNF-α (pg/ml)	Baseline	Mean	58.38	42.71	154.31	158.67
	Day 28	Mean	4.17	3.26	7.60	8.98
		% change	-93%	-92%	-95%	-94%
		P value	0.018*	0.014*	0.030*	0.057

4. Discussion

The current randomized, controlled study was conducted to assess the antigingivitis efficacy and safety of a novel antibiotic-free, cetylpyridium chloride, hydrogen peroxide-based formulation compared to with standard home care of brushing (with an antioxidant fluoride toothpaste) and flossing. This study also evaluated inhibitory properties of the test formulation on oral malodor as well as its teeth whitening efficacy. The mean GI changes indicated that the test formulation, when used with or without an accelerating device, significantly reduced gingivitis during a 6-week period. The mean BOP reductions seen in both the test groups further support the notion of the test formulation significantly reducing gingivitis, in that the test formulation applied with the accelerating device exhibited a sixteen-times greater effect of reducing gingival bleeding than brushing and flossing. As expected, the test formulation applied only on a toothbrush exhibited half of the bleeding reduction effect compared to using it with the device, but still an eight-times greater effect than brushing and flossing. The data also suggest that the test formulation is effective in the control of plaque supported by the finding of a statistically significant reduction in mean PI when using the test formulation with the device or on a toothbrush. Again, the test formulation applied with the device resulted in sixteen-times greater plaque reduction efficacy compared to brushing and flossing after a 6-week period. The test formulation is considered safe to use for the treatment and prevention of gingivitis, as no serious adverse events related to the study product(s) were reported for any participants, and no treatment related side effects such as irritation, staining of teeth or tissues, or taste alteration were reported. Further, the test formulation used in either application did not cause any adverse shifts in subgingival microflora during a 6-week period.

The efficacy of SRP, antibiotics and other periodontal therapies are well documented, however drawbacks exist with some treatments include the product of enamel defects, which cause teeth to appear stained[37]. Common antimicrobials used to treat periodontal infections, such as tetracycline, can permanently stain teeth, often turning them yellow or brown as the tetracycline molecule is deposited within the dental structure[38].

Research has suggested that microorganisms in patients with chronic periodontitis may be resistant to commonly used antibacterial agents[39]. The prominent periodontal pathogens, such as *P. gingivalis* and *A. actinomycetemcomitcans*, exhibit variable susceptibility to common antibiotics such as clindamycin, amoxicillin and metronidazole[39]. Providing dental practices and patients with alternative non-antibiotic methods to regulate bacterial biofilm, oral pH and inflammation is remarkably necessary as it may halt disease progression without undesired side effects or contribution to the global health crisis of antibiotic resistance.

In evaluating the primary endpoints of gingival redness and bleeding (BOP and GI), participants using the test formulation in either application (device or toothbrush) experienced statistically significant within- and between-treatment reductions in both parameters, well beyond that of the standard home care with or without flossing. When the test formulation was applied using the accelerating device, participants experienced on average, a sixteen-times greater reduction in gingival bleeding and plaque accumulation during a 6-week period compared to participants that brushed with an OTC fluoride toothpaste and flossed. When the test formulation was applied using a toothbrush, participants experienced half of the bleeding reduction effect than using the formulation with the device, however still eight-times greater efficacy than brushing and flossing. This result fully supports the trial hypothesis and is expected due to the nature of the test formulation. The test product is a post-foaming gel formulation, which increases in volume over time and with extrinsic energy input. The device in which the formulation was applied with is a proprietary universally sized mouthpiece that combines light and warming heat in a closed system to accelerate formulation breakdown and facilitate treatment efficacy – therefore, the two-times efficacy of the test

formulation when used with the device is expected as the warming heat of the mouthpiece is able to accelerate the foaming capacity of the gel, allowing optimal dispersion of the active ingredients throughout the oral cavity and into interproximal spaces where inflammation and periodontal diseases are most prevalent. In addition, the test formulation resulted in statistically significant within- and between-treatment reductions in GI (i.e., tissue tone & quality), indicating the formulation's utility in providing an at-home method to treat and stabilize mild to moderate gingival disease well beyond standard home care of brushing with a fluoride toothpaste and flossing.

Secondary outcomes assessed in the current study included changes from baseline in PI, PD, oral malodor and teeth whitening. Participants using the test formulation in either application experienced statistically significant plaque reductions indicated by decreases in PI score during the 6-week treatment period. Interestingly, participants who only brushed twice daily with the fluoride toothpaste saw significant plaque accumulation as indicated by the increase in PI score over the 6-week period. Further, the test formulation when used with the device was sixteen-times greater at reducing plaque levels compared to brushing and flossing. PI and GI scores are used as parameters to evaluate periodontal disease severity – an increase in plaque accumulation and a decline in gingival health indicate progression of disease[34]. The findings of the current study suggest that the test formulation is efficacious in controlling the progression of periodontal disease, as indicated by the significant reductions in PI and GI scores simultaneously, whereas the current standard of brushing and flossing has been shown here to not be adequate for stabilizing the periodontal condition between dental visits.

When analyzing the secondary outcome of change in PD from baseline to treatment endpoint, statistically significant changes occurred in Groups 1 and 2, however the reductions were not clinically relevant. This result is attributed to the homogeneity of the study population in terms of baseline mean PD. The study participants, similar to most of the U.S. population, had only mild gingivitis, which is why this parameter was included as a secondary outcome measure. When the sites were separated by disease severity, pockets characterized as "mild-moderate" (4-6mm) were reduced with statistical significance after 6-weeks across all groups.

A study conducted by Loe showed that gingivitis developed with refrainment from oral hygiene procedures, along with a change in bacterial composition of plaque[40]. As disease progresses and more harmful strains colonize, the oral bacterial balance changes from gram-positive aerobic to gram-negative anaerobic taxa[41], facilitated by a decreased oxidative environment[42]. Participants using the test formulation with the device (Group 1) experienced statistically significant bacterial count reductions during a 4-week period, particularly in the gram-negative periopathogenic taxa *P. gingivalis* and *A. actinomycetemcomitans*. Similarly, the test formulation applied with a toothbrush resulted in the significant reduction of the gram-negative bacteria, *P. gingivalis* and *T. denticola.* These bacteria are included in a category that Socransky and colleagues[36] have identified as "red complex bacteria", which have been strongly associated with periodontal disease[43]. The main pathogen for causing periodontitis in humans is *P. gingivalis*[44], and the test formulation when used with the device or on a toothbrush produced statistically significant reductions in the abundance of *P. gingivalis*, whereas using fluoride toothpaste with or without flossing did not result in significant reductions of any bacterial species analyzed. Of additional importance, *P. gingivalis* has been associated with several systemic inflammatory diseases including atherosclerosis[45], cardiovascular disease[46,47], stroke[46], colorectal[47], lung[48] and pancreatic[49] cancers, and research has indicated that the treatment of periodontal diseases (i.e., the reduction of "red complex" bacteria) can improve systemic inflammation[50].

GCF samples were analyzed for proinflammatory proteins (e.g., cytokines & chemokines) using multiplexing ELISA techniques. The proteins evaluated were IL-1β, IL-6, IL-8, MCP-1 and TNF-α at a picogram volume level. It is known that immunologic cascades following periodontitis induce the production of cytokines and the breakdown of epithelium and connective tissues[51], further exacerbating disease. Participants using the test formulation in either application (Groups 1 and 2) experienced statistically significant reductions in four of the five proteins analyzed. Both groups saw significant reductions in IL-6, IL-8 and TNF-α, while IL-1β was significantly reduced in Group 1 and MCP-1 significantly reduced in Group 2. IL-1β plays an important role in initiating and progressing periodontal inflammation, and have been found in higher levels in patients with periodontitis compared to healthy individuals[52]. In addition, these gingival inflammation-associated proteins have an implication on systemic disease in that IL-1β has effects on cells that make up atherosclerosis[53] and research has indicated that increased concentrations of serum cytokines are associated with periodontitis patients[54]. Of further importance, research of the association between periodontal disease and inflammatory markers, such as C-reactive protein, interleukins and s-PLA2, have indicated the systemic benefits of periodontal therapy[24]. The finding of the test formulation and device producing significant reductions in IL-1β is supportive of the changes seen in periopathogenic bacterial load, plaque abundance, inflammation and gingivitis condition, representing a promising approach to managing disease progression and maintenance beyond brushing and flossing. It is evident that patients need a better way to control plaque and inflammation at home that can be adjunctive to therapy administered in a dental practice.

Tooth color evaluation by comparison of a participant's tooth with a shade guide is the most frequent methodology in clinical dentistry in assessing tooth color measurement changes[55]. Although this method is considered highly subjective, computerized determinations are also subject to errors[56] and porcelain shade guides remain the most common shade taking method[57]. In addition, there was a single examiner assessing color shade, to avoid inter-examiner error. Participants using the test formulation with the device (Group 1) experienced statistically significant within-treatment changes from baseline in their tooth color of 0.5 shades after 5 days (p=0.030) and a full shade by the end of the 6-week treatment (p=0.007), on average. This shade change was maintained during the 2-week period that test formulation use was discontinued. Participants using the fluoride whitening toothpaste with or without the test formulation experienced roughly two-thirds of a shade change after 6-weeks, however the differences did not reach statistical significance (p_2=0.18; p_3=0.06).

The final secondary endpoint evaluated was the change in oral malodor from baseline to 5, 42 and 60 days. Three VSCs were evaluated at the ppb level – H_2S, CH_3SH and $(CH_3)_2S$. Participants using the test formulation with the device (Group 1) experienced statistically significant reductions in one of the three VSCs analyzed at days 5 (CH_3SH), 42 (H_2S) and 60 (H_2S). Differences in VSC concentration for all other groups were not found to be statistically significant. It is important to note that the study population were not diagnosed with halitosis and all presented, on average, with "normal" baseline VSC concentrations (<180pbb) as indicated in the literature[58]. However, the test formulation demonstrated breath freshening ability when administered by the described protocol.

5. Conclusion

It is well established that the majority of American adults live with some degree of periodontal disease, with the highest prevalence among the elderly. In lieu of the Cochrane review that demonstrated weak, very unreliable evidence suggesting that flossing with toothbrushing may be associated with small plaque reduction at 1 or 3 months[59], it is still assumed by those in the dental profession, that proper flossing will control plaque accumulation and help to prevent gum disease and tooth decay. It is known that roughly 13% of the U.S. claim to floss regularly[60], and while this incompliance may be due to technique sensitivity or to general poor habit, it is important to recognize the negligence that the population has towards oral hygiene and how that relates to the current periodontal disease status in the U.S. By providing patients nationwide with an innovative method of controlling gingival inflammation, bleeding, plaque and bacterial balance that is simple to incorporate into a daily hygiene routine and void of typical treatment compromises (e.g., the test formulation gently whitens teeth and freshens breath), may aid in shifting the population ratio of periodontal disease and in turn potentially influence several systemic inflammatory diseases.

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