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Essential oils in one-stage full-mouth disinfection: double-blind, randomized clinical trial of long-term clinical, microbial and salivary effects

Sheila Cavalca Cortelli[1], José Roberto Cortelli[1], Marinella Holzhausen[1], Gilson Cesar Nobre Franco[2], Renato Zanotta Rebelo[3], Alan Salinas Sonagere[3], Celso da Silva Queiroz[2] and Fernando Oliveira Costa[4]

[1]Department of Periodontology, University of Taubaté, São Paulo, SP, Brazil; [2]Department of Oral Biology, University of Taubaté, São Paulo, SP, Brazil; [3]Dental School, University of Taubaté, São Paulo, SP, Brazil; [4]Department of Periodontology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

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Abstract

Aim: This randomized clinical trial evaluated the effects of an essential oils-containing mouthrinse for full-mouth disinfection.

Material and Methods: Fifty patients were assigned to receive full-mouth disinfection with either essential oils or placebo. At baseline, 2 and 6 months of treatment the primary outcomes probing depth (PD), plaque index (PlI) and modified gingival index (MGI) were monitored. Additional monitoring included bacterial presence (by polymerase chain reaction) in subgingival, saliva and tongue samples; flows, pH, total protein and alkaline phosphatase salivary levels. The following statistics were used: ANOVA, Student's *t*-test, χ^2 and Kruskal–Wallis ($p<0.05$).

Results: Mean PD $\geqslant$ 3.5 mm was reduced over time in both the placebo and the test groups, but there was no difference in PD reduction between groups at 2 and 6 months. At 2 and 6 months, PlI and MGI showed greater reductions in the test group than in the placebo group. *Porphyromona gingivalis* was not reduced in any site. At 6 months, *Campylobacter rectus* increased in both groups, while *Tannerella forsythensis* decreased subgingivally in the test group. *S. sanguinis* increased, except subgingivally, in the placebo group. Salivary pH and flows were not altered. Total protein reduced only in the test group. Alkaline phosphatase did not change in either group.

Conclusions: Essential oils for full-mouth disinfection showed clinical benefits, namely reducing plaque and gingival inflammation without altering basic salivary parameters.

Key words: clinical trials; essential; full-mouth disinfection; oils; periodontitis; therapy

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Conflict of interest and source of funding statement

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Many oral diseases are plaque related. Dental plaque is a microbial biofilm that is formed by organisms tightly bound to a solid substrate and to each other by means of an exopolymer matrix. Physical, metabolic and physiological interactions can contribute to the changes in the microbial composition of the biofilm that are observed in the progression from a healthy oral environment to periodontal disease. As such, periodontal therapy aims to reduce periodontal pathogens and increase the presence of beneficial bacterial species.

Traditionally, in a conservative periodontal treatment, scaling and root planing is often performed over the course of 2–3 months (one quadrant or sextant at a time with a 1–2-week interval between clinical appointments). However, although this method has a well-documented success rate (Badersten

et al. 1981, Hämmerle et al. 1991), this standard strategy seems to allow for rapid recolonization and intra-oral bacterial translocation from untreated sites to recently disinfected sites (Van der Velden et al. 1986, Sbordone et al. 1990).

To avoid this rapid colonization, Quirynen et al. (1995) proposed a one-stage full-mouth disinfection protocol in which mechanical therapy is performed within 24 h in conjunction with the full-mouth application of chlorhexidine. Although a few reports failed to find greater improvements with this method (Apatzidou & Kinane 2004, Apatzidou et al. 2004) when compared with the standard quadrant-by-quadrant treatment, one-stage full-mouth disinfection seems to provide satisfactory clinical (Quirynen et al. 1995, 2000, Vandekerckhove et al. 1996, Bollen et al. 1998, Lang et al. 2008, Sanz & Teughels 2008) and microbiological results (Quirynen et al. 1995, 1999, 2000, Bollen et al. 1996, 1998, De Soete et al. 2001, Lang et al. 2008).

Quirynen et al. (2006a) reported that these additional benefits could be partially explained by the mechanical procedures being conducted over a short time period and by the chemical agent selected. More recent studies have focused on modifications of the original protocol such as full-mouth scaling and root planing in conjunction with azithromycin treatment (Gomi et al. 2007) or one-stage periodontal debridement with an ultrasonic instrument in combination with 0.5% povidone (pvp)-iodine (Zanatta et al. 2006).

Essential oils possess anti-plaque and anti-gingivitis properties (Charles et al. 2001, 2004, Witt et al. 2005, Patel & Malaki 2008) as demonstrated by their action against oral microorganisms (Fine et al. 2007a, b) with only minimal side effects. Therefore, it seems reasonable to evaluate whether essential oils work for one-stage full-mouth disinfection. Cortelli et al. (2008a) conducted a preliminary study and found promising clinical results in patients with generalized moderate chronic periodontitis who received scaling and root planing in 24 h in combination with treatment with essential oils and rinsing twice a day for 15 days with the same essential oils.

Many salivary components represent useful biochemical markers that could offer a cost-effective, non-invasive approach for monitoring the health–disease process in the mouth. Various oral antiseptics contain alcohol, and the mouthrinse tested contains 21.6% ethanol USP. There is a concern related to any possible relationship between the alcohol, as well as the low pH of oral products, and oral dryness, epithelial desquamation and oral cancer. Unfortunately, a systematic review of the effects of full-mouth disinfection with and without antiseptics (Lang et al. 2008) did not identify randomized clinical trials that examined salivary parameters.

Therefore, in the present study, we report the results from a randomized, double-blind placebo-controlled clinical trial conducted to evaluate whether a mouthrinse containing essential oils would result in unaltered basic salivary parameters, while providing clinical or microbial benefits. The patient population studied had mild periodontitis treated with ''one-stage full-mouth disinfection'' and who rinsed with this product for 2 months.

Materials and Methods

Study design

It was hypothesized that one-stage full-mouth disinfection performed in conjunction with the long-term use of a mouthrinse containing essentials oils would provide additional oral benefits without altering the pH and salivary flow in the mouths of mild periodontitis patients. Therefore, we investigated, in a randomized, double-blind, placebo-controlled clinical trial, the long-term clinical, microbial and salivary effects of a mouthrinse containing essential oils used for a one-stage full-mouth disinfection.

All patients of both genders requesting periodontal treatment at the Dental clinic, University of Taubaté, SP, Brazil, who were between 30 and 50 years of age with at least 15 teeth were candidates for inclusion (from September 2006 to February 2007) in the study. Inclusion criteria for this study were two periodontal pockets in each quadrant with PD of 4 mm and, at maximum, four periodontal pockets of 5 mm; clinical attachment loss (CAL) $\leqslant 2.5$ and $\geqslant 1.5$ mm; and a mean modified gingival index (MGI) of 1.75 or greater. Exclusion criteria were: (1) no diagnosis of mild generalized chronic periodontitis or diagnosis of any type of gingival overgrowth, (2) any furcation lesions, (3) current or former smoking, (4) diabetes and/or immunological disease, (5) pregnancy or breast-feeding, (6) subjects wearing orthodontic devices, extended prosthetic devices or having overhang restorations, (7) periodontal treatment 12 months before the beginning of the study, (8) use of local/systemic antibiotics within the past 6 months, (9) a need for antibiotics prophylaxis, (10) routine use of mouthrinse in the previous 6 months, (11) alcohol abuse and (12) unwillingness to return for follow up.

The desired sample size of 20 subjects per group was based on the results of a preliminary study (Cortelli et al. 2008a) and was calculated to provide 90% power ($\alpha = 0.05$) to detect a 0.5 mm between-group difference in PD. We included five additional subjects per group to account for the estimated dropout generally observed in longitudinal studies.

Data and personal information related to the medical and dental histories were obtained by a questionnaire. All subjects signed an informed consent form, which was previously approved by the Committee on Research Involving Human Subjects (protocol number 328/06) from University of Taubaté.

Clinical examinations

A complete periodontal examination was carried out. Measurements of PD (PCPUNC 15 Hu-friedy Mfg Co Inc., Chicago, IL, USA), plaque index (PlI) (PCPUNC 15 Hu-friedy Mfg Co Inc.) (Silness & Löe 1964) and MGI (Lobene et al. 1986) were obtained by one blinded, trained and calibrated examiner as described previously (Araujo et al. 2003) in six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual). The examiner was considered calibrated if the standard error of measurement (SEM) for PD was <0.8 mm and if κ was >0.80 and <0.95 for MGI. Intra-examiner error was recalculated 1 week before the 6-month evaluation.

Microbiological examinations

Microbial samples were obtained as reported previously (Cortelli et al. 2005). Briefly, eight periodontal sites, two in each quadrant (PD $\geqslant 4$ mm associated with bleeding on probing and CAL), were selected for each subject. Each selected tooth was isolated with sterile cotton rolls and the supragingival plaque was removed with sterile cotton

pellets. A sterilized paper point (number 30) was carefully inserted to the depth of the periodontal pocket, and kept in position for 60 s. The pooled subgingival samples were stored at −80°C in microtubes containing 1 ml of reduced Ringer's solution.

Microbial samples from the dorsum of the tongue were obtained from areas of approximately 1 cm^2, using a swab with reduced Ringer's solution, which was rotated six times. Each swab was placed in a microtube containing 1 ml of reduced Ringer's solution. Samples of non-stimulated saliva were also collected in sterile tubes. Immediately after collection, 0.1 ml of whole saliva was diluted in 1 ml of reduced Ringer's solution.

The presence of *Campylobacter rectus*, *Porphyromonas gingivalis*, *Tannerella forsythensis* and *Streptococcus sanguinis* was established by a polymerase chain reaction (PCR) using specific primers [*P. gingivalis,* sense: 5′-AGGCAGCTTGCCATACTGCGG3′-, and antisense: 5′-ACTGTTAGCAACTACCGATGT-3′ (product size: 404 bp); *T. forsythensis*, sense: 5′-GCGTATGTAACCTGCCCGCA-3′, and antisense: 5′-TGCTTCAGTGTCAGTTATACCT-3′ (product size: 641 bp); *C. rectus*, sense: 5′-TTTCGGAGCGTAAACTCCTTTTC-3′, and antisense: 5′-TTTCTGCAAGCAGACACTCTT-3′ (product size: 598 bp) and *S. sanguinis*, sense: 5′-GAAGCCATTTTGCCTAGATTGATGG-3′ and antisense: 5′-CCATACCGATTCCTTACTCTAAATTT-3′ (product size: 475 bp)] under standard conditions. The DNA was extracted using InstaGene Matrix (BioRad Laboratories, Hercules, CA, USA) and the PCR was performed in a Mastercycler Gradient (Eppendorf®, Westbury, NY, USA) thermocycler as follows: one cycle 94°C for 5 min., 35 cycles 94°C for 30 s, 55°C for 30 s, 72°C for 1 min., and a final extension of 72°C for 5 min.

After electrophoresis in 1.5% agarose gel, the DNA fragments were stained with SYBR Safe™ (Invitrogen®, Carlsbad, CA, USA) and visualized by UV illumination. The PCR amplificates were compared with both positive and negative controls. A molecular weight marker (Ladder 100) was added in each set.

Salivary examinations

Saliva samples (unstimulated and stimulated) were collected from the volunteers between 09:00 and 11:00 hours to avoid circadian rhythm effects. No food or drink was permitted for 2 h before collection. During the sample collection, the volunteers remained in a seated position, with their head tilted forward (approximately 45°). The procedure was accomplished in a quiet and well-ventilated room. Initially, the examiner instructed each individual to collect unstimulated whole saliva produced during a 1-min. period. This first sample was discarded and afterward, a second sample was collected over a 5-min. period into a preweighed plastic tube (Navazesh et al. 1992).

After an interval of 5 min., the volunteers were instructed to chew a Parafilm® (São Bernardo do Campo, Brazil) block for 5 min. The examiner asked the individuals to spit out saliva each minute. The first 2 min. of collection were discarded, and so the analyses were performed using the last three collections (Navazesh et al. 1992).

Immediately after collection, the salivary pH was measured using a portable pH meter (Marconi P200, São Paulo, Brazil). Its glass electrode was washed with deionized water and calibrated with buffer solutions pH 7.0 and 4.0.

Additionally, the salivary flow rate, which is defined as the total volume of saliva produced per unit time (ml/min.), was determined considering the volume of saliva (ml) as the difference between the weight (g) of the plastic tube before and after collection. The density of saliva was considered to be 1.0 (Navazesh 1993). A stimulated salivary flow rate between 1.0 and 3.0 ml/min. was considered normal, while values <0.7 ml/min. indicated a reduced flow rate. For unstimulated saliva, values between 0.3 and 0.5 ml/min. were considered as normal flow (Krasse 1988).

Before biochemical analyses, the saliva samples were centrifuged at 12,000 r.p.m. for 5 min. and the resulting supernatant was collected and used for the following analyses. Total protein quantification was performed using the Biuret method using a specific analytical kit (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil). This method is based on the reaction between peptide bonds of the protein and Cu^{2+}, which produces a blue-violet coloured complex. The absorbance at a wavelength of 540 nm was read using a spectrophotometer. Salivary phosphatase alkaline quantification was also performed using a colourimetric commercial kit (Gold Analisa Diagnóstica), with absorbance measured at 590 nm.

Periodontal therapy

Participants with mild periodontitis were randomly allocated to receive either one-stage full-mouth disinfection plus essential oils/Listerine® (Guarulhos, Brazil) cool mint (test group) or one-stage full-mouth disinfection plus placebo (control group) as detailed in Table 1. The pharmacy Byofórmula (São José dos Campos, SP, Brazil) produced the placebo solution (Sorbitol solution 15%; ethanol USP 21.6%; sodium saccharin 0.05%; benzoic acid 0.1%; mint flavouring QS; sodium benzoate; dye green QS; and water QSF 1 l) according to the Listerine® cool mint formula, except for active agents (thymol 0.064%; menthol 0.042%; eucalyptol 0.092%, and methyl salicylate 0.06%).

One independent specialist in pharmacology dispensed 240 ml of either essential oils or placebo in identical bottles numbered with the randomization code on the label, but no other identifying information, according to a computer-generated randomization list provided by the statistician. A research-

Table 1. One-stage full-mouth disinfection plus a mouthrinse containing essential oils or placebo as conducted in the present study

Full-mouth scaling and root planing (the entire dentition in two visits within 24 h, i.e. two consecutives mornings) under local anaesthesia
Polishing of the treated quadrants with abrasive paste
Friction twice (at the beginning and at the end of each visit) of the dorsum of the tongue by rubbing with a sterilized cotton swab soaked with 0.2 ml of essential oils or placebo for 1 min.
Mouth rinsing twice (at the beginning and at the end of each visit) with 20 ml of essential oils or placebo mouthrinses for 30 s (during the last 10 s, the subject had to gargle)
Subgingival irrigation of all pockets (PD $\geqslant$ 4 mm) three times within 10 min. with essential oils or placebo mouthrinses (5 ml/irrigation/pocket) after both sessions of scaling and root planing. This was repeated on day 8
Mouth rinsing at home with 20 ml of essential oils or placebo mouthrinses twice daily for 30 s for the following 2 months
Oral hygiene instructions including tooth brushing, flossing or inter-dental cleaning with inter-dental brushes and tongue brushing

PD, probing depth.

er responsible for seeing the periodontal patients allocated the next available number upon the patient's entry into the trial. Participants and researchers who had any contact with these participants were blinded to treatment assignment for the duration of the study.

Before packaging the placebo in Listerine® bottles, these containers were washed with distilled water five times a day (10 min. each) for 3 consecutive days. After 6 days, each participant received a new bottle with the same volume as before. All participants also received cups with a mark to indicate a 20 ml volume. The initial rinse was performed under supervision at the study centre, and the remaining rinses were performed unsupervised at home.

Active periodontal treatment was provided between March 2007 and July 2007. Scaling and root planing was performed with Gracey and McCall curettes and Hirschfield periodontal files by the same trained periodontist. Periodontal scalers were discarded after six uses. The time required for each quadrant was approximately 1 h and quadrants I–IV were treated clockwise. Every month, each subject received a standard kit for mechanical supragingival plaque control containing fluoride dentifrice (Colgate Tripla Ação, Colgate, Osasca, Brazil), a toothbrush (Reach – Professional Extreme 3, Johnson & Johnson, Morris Plains, NJ, USA), interdental toothbrushes (Conical, Oral B, São Paulo, Brazil) and dental floss (Reach – expansion plus, Johnson & Johnson).

Patients were encouraged to comply with the study protocol. Compliance, desirable and undesirable side effects were evaluated by a questionnaire. Participants were reminded daily to rinse twice a day (morning and evening) via a telephone call for the first 60 days. Additional phone calls were made every 15 days for up to 6 months.

At baseline (T0), 2 (T1) and 6 (T2) months, the examiners collected the clinical, microbial and salivary data.

Statistical analysis

The primary endpoint with respect to benefits in one-stage full-mouth disinfection was the reduction in the mean values of pocket depth, the amount of plaque present and gingival inflammation from baseline to 6 months and any difference between the essential oils and the placebo groups. Additional analyses were performed to evaluate changes in bacterial prevalence. Finally, the lack of changes in pH and salivary flow was considered a positive outcome as was a reduction in both total protein and alkaline phosphatase levels.

Intention-to-treat analyses with the Last Observation Carried Forward were performed. Therefore, 50 subjects from the baseline visit (25 per group) were included in our intention-to-treat analysis. All collected data from the non-compliant placebo-group subject and the subject who started smoking after the 2-month visit were included in the statistical analysis. Moreover, for the patient who moved to another city, the 6-month missing values were substituted by their respective last observations.

All statistical analyses were carried out using SPSS® 11.5, SAS 9.1.3 and Biostat 5.0. Initially, all data were tested for normality using D'Agostinho-Pearson test. After that, the between-group homogeneity was tested. Baseline data presented a normal distribution and did not show between-group differences. Because of the lack of differences, clinical data were analysed using repeated-measures ANOVA and Student's *t*-tests. Microbial data were analysed by applying a χ^2 test while salivary data were analysed using a Kruskal–Wallis test. Differences were considered statistically significant when $p < 0.05$.

Results

Figure 1 shows the flow of participants throughout the study. Regarding baseline demographic characteristics, the mean age ± SD (years) was 40.68 ±

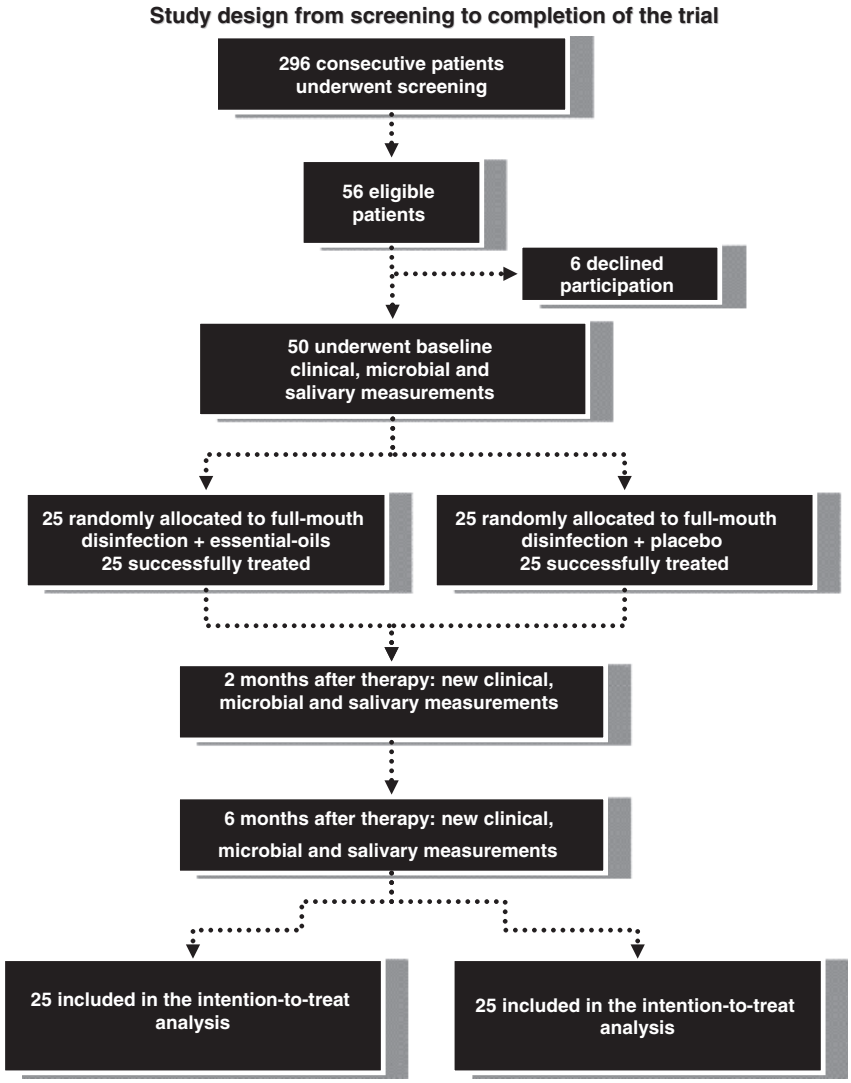

Fig. 1. Study design since screening phase until completion the trial.

7.04 for the test group and 41.22 ± 6.71 for the placebo group and the male:female ratios were 12:13 and 4:11, respectively. Eligible participants were recruited from December 2006 to February 2007. Participants attended monitoring clinic visits at baseline and at 2 months and at 6 months after the initial therapy in addition to visits related to periodontal treatment. Two patients (8%) from the test group and one (4%) from the placebo group did not complete properly the full 6 months of follow-up. In the present study, we performed the primary analysis as intention to treat involving all participants. Therefore, the subject who started smoking (test group) and the non-compliant subject (placebo group) were included in the analysis. For the patient who moved to another city we adopted the Last Observation Carried Forward strategy. Patients did not report adverse events that could be either therapy or drug related.

Clinical results

Aiming to evaluate the clinical effects of the tested therapeutic protocol, we chose to examine three periodontal parameters, PD, PlI and MGI. The mean values for these parameters are shown in Table 2. At baseline, both groups showed the same pattern and severity of periodontal disease revealed by the lack of statistically significant differences between groups in any of the clinical parameters. There was no statistically significant difference in overall mean PD for the placebo group at 2 and 6 months compared with baseline, but overall mean PD in the test group was significantly less ($p < 0.05$) at 2 and 6 months than at baseline. However, comparisons between the test and placebo group at each time point did not reveal significant differences between groups. Table 3 gives statistical comparisons over time and between groups in each time point for mean PD in two categories (PD < 3.5 and PD > 3.5 mm). Between-group analysis at each time point did not reveal significant differences. Differences from baseline at 2 and 6 months were statistically significant ($p < 0.05$) for shallow pockets in the test subjects and for deep pockets at 2 and 6 months for both test and placebo groups. No differences were found in mean PD between 2-month and 6-month time periods for any group.

Table 2. Intention-to-treat analysis of comparisons over time of clinical parameters between test ($n = 25$) and placebo ($n = 25$)

Clinical parameter	Evaluation	(T0) baseline	(T1) 2 months	(T2) 6 months
PD	Test	3.51 ± 1.34 A a	2.55 ± 0.71B a	2.5 ± 0.75 B a
Mean ± SD	Placebo	3.52 ± 1.43 A a	3.02 ± 1.22 A a	2.97 ± 1.22 A a
PlI	Test	2.11 ± 0.61 A a	0.48 ± 0.41 B a	0.52 ± 0.43 B a
Mean ± SD	Placebo	2.12 ± 0.63 A a	1.23 ± 0.68 B b	1.3 ± 0.66 B b
MGI	Test	2.74 ± 0.23 A a	0.47 ± 0.36 B a	0.56 ± 0.39 B a
Mean ± SD	Placebo	2.52 ± 0.55 A a	1.39 ± 0.62 B b	1.48 ± 0.66 B b

Intra-group (different capital letters within the same line) and inter-groups (different lower-case letters within the same column for each clinical parameter) analysis revealed significant differences (ANOVA and Student's *t*-test, $p < 0.05$).
PD, probing depth; PlI, plaque index; MGI, modified gingival index; SD, standard deviation.

Regarding the PlI, intra-group analysis showed a reduction in both groups (T0 > T1 = T2). Furthermore, inter-group comparisons demonstrated greater reductions for the test group at the 2- and 6-month evaluations. Interestingly, MGI showed intra- and inter-groups profiles of reduction similar to PlI. Results from PII and MGI are also shown in Table 2.

Microbial results

We evaluated the frequency of three periodontal pathogens and one beneficial bacterial species in samples collected from patients' subgingival biofilm, unstimulated whole saliva and the dorsum of the tongue. Only intra-group analyses demonstrated significant differences (Table 4).

P. gingivalis was not reduced in any sampled site. This bacterium increased at 6 months in saliva samples from the placebo group. Furthermore, a tendency to increase for both groups was observed from baseline to 2 months. *C. rectus* at 6 months increased in all sampled sites for both groups (Table 4). *T. forsythensis* reduced for test groups at 6 months, and increased at 2 months in saliva and tongue samples for the placebo group. Changes in the prevalence of *S. sanguinis* were similar for both groups, except for the periodontal pockets in the placebo group, which showed increases from baseline to 2 months that were not observed in the test group.

Salivary results

The tested mouthrinse containing essential oils, when used in the one-stage full-mouth disinfection, did not alter the pH or flow of saliva (Table 5). Moreover, only the test group showed

Table 3. Intention-to-treat analysis ($n = 25$ per group) of mean probing depth between test and placebo groups over time in two pocket depth categories (PD < 3.5 and PD ⩾ 3.5 mm)

PD category	(T0) baseline	(T1) 2 months	(T2) 6 months	*p*-value		
				baseline × 2 months	baseline × 6 months	2 months × 6 months
< 3.5 mm						
Test	2.36 ± 0.33	2.04 ± 0.28	1.94 ± 0.28	0.0145	0.0019	0.4324
Placebo	2.26 ± 0.35	2.08 ± 0.37	2.02 ± 0.40	0.2744		
Test × placebo, *p*-value	0.4980	0.7680	0.5635	NA	NA	NA
⩾ 3.5 mm						
Test	4.88 ± 0.45	3.16 ± 0.55	3.17 ± 0.54	0.0001	0.0001	0.9628
Placebo	4.89 ± 0.64	4.03 ± 0.96	4.01 ± 0.92	0.0253	0.0216	0.9449
Test × placebo, *p*-value	0.9600	0.0750	0.1162	NA	NA	NA

Data analysed by ANOVA and Student's *t*-test (post hoc test).
PD, probing depth; NA, not applicable.

Table 4. Intention-to-treat analysis of prevalence changes of *Porphyromona gingivalis*, *Campylobacter rectus*, *Tannerella forsythensis* and *Streptococcus sanguinis* for test ($n = 25$) and placebo ($n = 25$) groups considering the sampled sites and examination time points

	Time points		
	baseline	2 months	6 months
P. gingivalis			
Periodontal pocket			
Test	23.53	47.37	33.33
Placebo	21.05	38.10	42.11
Tongue dorsum			
Test	17.65	31.58	27.78
Placebo	27.78	45.45	21.05
Saliva			
Test	42.86	47.37	33.33
Placebo	53.33(A/B)	37.78 (A)	70.00 (B)
C. rectus			
Periodontal pocket			
Test	29.41(B)	63.16(A)	83.33(A)
Placebo	47.37(B)	66.67(A/B)	84.21(A)
Tongue dorsum			
Test	64.71(B)	73.68(A/B)	100.00(A)
Placebo	66.67 (B)	72.09 (A/B)	89.47 (A)
Saliva			
Test	64.29(B)	57.09(B)	100.00(A)
Placebo	60.00(B)	70.00(A/B)	94.44(A)
T. forsythensis			
Periodontal pocket			
Test	68.42(A)	61.90(A)	31.58(B)
Placebo	41.18	57.89	33.33
Tongue dorsum			
Test	41.18	68.42	44.44
Placebo	33.33(B)	72.73(A)	36.84(B)
Saliva			
Test	50.00	63.16	44.44
Placebo	38.89 (B)	70.00 (A)	50.00 (B)
S. sanguinis			
Periodontal pocket			
Test	16.67(B)	52.94(A)	57.89(A)
Placebo	21.05(B)	21.05(B)	52.38(A)
Tongue dorsum			
Test	38.89(B)	70.59(A)	73.68(A)
Placebo	42.11(B)	77.78(A)	54.55(A/B)
Saliva			
Test	55.56(B)	92.86(A)	78.55(A/B)
Placebo	27.78(B)	86.67(A)	55.00(A/B)

Intra-group analysis revealed significant differences among time points (different capital letters within the same line; χ^2-test, $p < 0.05$) while inter-group analysis did not (χ^2-test, $p > 0.05$).
The prevalence values are based on the number of subjects with detectable bacterial species.

a reduction in total protein levels. Levels of alkaline phosphatase were not influenced by this kind of periodontal therapy.

Discussion

Although scaling and root planing result in periodontal improvements, the field of periodontics still seeks a well-tolerated therapeutic protocol that also produces sustained clinical and microbial changes. One-stage full-mouth disinfection shows good acceptance by periodontal patients if it is conducted for a short time, which is an important aspect for busy people.

Essential oils have a 125-year history of usage, well-documented anti-plaque and anti-gingivitis properties and are easily incorporated into a daily oral care routine. Because these two treatment paradigms seem to both have positive effects, it seems appropriate to combine one-stage full-mouth disinfection with essential oils to treat chronic periodontitis patients, especially considering the worldwide prevalence of this type of disease. This paper focused on clinical, microbial and salivary long-term effects associated with mechanical procedures performed in a short time period and in conjunction with a mouthrinse containing essential oils.

Quadrant-by-quadrant periodontal treatment reduces periodontal pathogens (Ali et al. 1992), however, the process is time consuming and seems to be followed by a rapid pathogenic recolonization (Van der Velden et al. 1986, Sbordone et al. 1990). One-stage full-mouth disinfection has shown promising results in preventing this recolonization when compared with the standard protocol. Bollen et al. (1996), in a 8-month study, reported better improvements for gingival index, PlI, PD and gingival recession among subjects from one-stage full-mouth disinfection. Later, Mongardini et al. (1999) presented similar findings after treating chronic and aggressive periodontitis patients.

In the present study, the test group showed a PD reduction from baseline to 2 months in comparison with the placebo group. This reduction remained unchanged until the end of the experimental period. However, between-group comparisons did not reveal any significant difference, most likely due to the tendency towards improvement observed in the placebo group. In contrast, in a preliminary study conducted by our group (Cortelli et al. 2008a), we observed significant differences between test and placebo groups. Although no differences were found between the mean PD values in the test *versus* placebo group at any time period for either category of baseline probing depths ($PD < 3.5$ or $PD \geqslant 3.5$ mm), the mean depth of shallow pockets in test subjects were significantly reduced from baseline at 2 and 6 months and the mean depth of deep pockets in both test and placebo subjects were significantly reduced from baseline at 2 and 6 months. According to Müller (2007), the deeper a periodontal pocket is, the better the expected therapeutic result should be in terms of PD reduction. Then, as in the preliminary study, we included patients with more severe periodontitis (moderate chronic periodontitis), they showed higher baseline PD values (test: 4.90 ± 0.47 mm; placebo: 5.02 ± 0.51 mm) compared with the baseline values found in the current study (test: 3.51 ± 1.34 mm; placebo: 3.52 ± 1.43 mm). These differences could contribute to the more evident

Table 5. Intention-to-treat analysis of salivary parameters comparisons between test ($n = 25$) and placebo ($n = 25$) groups considering the examination time points

	Examination time points, mean ± SD		
	baseline	2 months	6 months
Unstimulated salivary flow (ml/min.)			
Test	0.38 ± 0.15	0.30 ± 0.14	0.31 ± 0.16
Placebo	0.38 ± 0.16	0.33 ± 0.12	0.45 ± 0.21
Stimulated salivary flow, ml/min.			
Test	2.1 ± 0.72	2.0 ± 0.53	2.1 ± 0.42
Placebo	2.3 ± 0.62	2.05 ± 0.59	2.2 ± 0.55
pH			
Test	7.16 ± 0.36	7.31 ± 0.23	7.15 ± 0.33
Placebo	7.25 ± 0.55	7.19 ± 0.21	7.17 ± 0.33
Total protein (mg/ml)			
Test	697.07 ± 142.36^{B}	758.22 ± 241.22^{B}	629.95 ± 132.17^{A}
Placebo	679.77 ± 130.34	981.86 ± 241.06	654.31 ± 116.80
Alkaline phosphatase (UI/l)			
Test	62.30 ± 8.63	66.42 ± 6.32	63.10 ± 8.82
Placebo	62.29 ± 10.20	69.51 ± 6.07	63.62 ± 7.35

Intra-group analysis revealed significant reduction from 2 to 6 months (different capital letters within the same line; Kruskal–Wallis test, $p < 0.05$) while inter-group analysis did not (Kruskal–Wallis test, $p > 0.05$).

PD changes observed in the preliminary research.

The most important clinical improvements occurred from baseline to 2 months. Despite both groups showing improvements in PlI and MGI, treatment with essential oils resulted in greater reductions than placebo treatment. Therefore, our results corroborate studies that showed the anti-plaque and anti-gingivitis effects of essential oils (Mendes et al. 1995, Charles et al. 2001, 2004, Witt et al. 2005, Gunsolley 2006, Patel and Malaki 2008). Furthermore, it is important to emphasize that even with cessation of mouthrinse use the PlI and MGI mean values observed at 6 months were close to those observed at 2 months, suggesting the residual effect of the essential oils in the test group. As previously reported for gingivitis patients (Sharma et al. 2004), the residual effects observed at 6 months in our periodontitis patients were probably facilitated by mechanical plaque self-control.

Our clinical findings are in agreement with the paper published by Quirynen et al. (2006a), which attributes the benefits of one-stage full-mouth disinfection to both short-term conduction and the antimicrobial actions of the chemical agent. A few authors, such as Apatzidou & Kinane (2004), did not observe clinical improvements after full-mouth disinfection. However, according to Quirynen et al. (2006b), we need to keep in mind that some discrepant results reported in the literature pertaining to one-stage full-mouth disinfection can be explained by major differences in the evaluated treatment protocol. In fact, some researchers failed to properly reproduce the original treatment protocol (Quirynen et al. 1995).

Although studies that tested either one-stage full-mouth disinfection with chlorhexidine (Bollen et al. 1998, Quirynen et al. 1999) or essential oils in different clinical conditions (Charles et al. 2000, Witt et al. 2005, Albert-Kiszely et al. 2007, Fine et al. 2007a, b) did find good antimicrobial effects, in the present study, *T. forsythensis* was only reduced subgingivally at 6 months in the test group whereas *P. gingivalis* did not show any reduction in these samples. The amount of this last bacterium increased at 6 months in saliva samples from the placebo group. Actually, a tendency towards an increase in *P. gingivalis* was observed for both groups. Our results differ from De Soete et al. (2001), who reported an additional benefit from one-stage full-mouth disinfection regarding *P. gingivalis* reduction, and from Fine et al. (2007b), who found a 66.3% reduction in *P. gingivalis* counts using a culture methodology.

At 6 months, *C. rectus* increased at all sampled sites for both groups. Despite its uncertain definition as a periodontal pathogen (Renvert et al. 1996) and its presence in periodontally healthy patients (Cortelli et al. 2008b), and considering that *C. rectus* is a member of the orange complex, which precedes colonization by more pathogenic species (Socransky et al. 1998), this is not considered a favourable result. Van der Weijden et al. (1998) conducted an in vitro study to establish the inhibitory effect of an herbal extract mixture on a select number of micro-organisms and to test, in vivo, the effect of a mouthwash containing 6.3 mg/ml herbal extract mixture on plaque formation and gingivitis development. The authors used a mouthrinse lacking active ingredients as a control and they did not observe any effect of the herbal extracts against *C. rectus*.

The *S. sanguinis* increases were similar between groups, except for the subgingival sample in the placebo group (from baseline to 2 months). This shift was also observed with the implementation of another therapeutic protocol (Carvalho et al. 2005), and seems to be a favourable result because *S. sanguinis* is considered to be a beneficial periodontal bacterial species that, through competition, limits periodontopathogen colonization (Kawashima et al. 2003, Van Hoogmoed et al. 2008).

Because the limit of detection of any laboratory technique influences results, the microbial findings of the present study need to be considered in conjunction with clinical improvements. Moreover, subject-based prevalence observed in the present study could also influence differences among the tested periodontopathogens. Further studies should be conducted to test whether the additional use of a mouthrinse containing essential oils between the third and sixth months post-treatment impacts microbial populations.

To screen for undesirable side effects related to extensive 60-day continuous use of the tested product, salivary pH and stimulated and unstimulated salivary flow were monitored beginning at baseline for 6 months. In the present study, the mouthrinse containing essential oils did not alter pH or salivary flows. According to Sahingur & Cohen (2004), appropriate salivary flow is a key factor in the maintenance of oral homeostasis. Kerr et al. (2007) did not observe differences in subjective and objective measurements of oral dryness applied to determine the influence of alcohol-containing mouthrinses in the salivary flow of non-xerostomic subjects. In 2003, after reviewing the literature, Claffey noted that the previous studies did not observe pH changes resulting from the use of essential oils.

Moreover, total protein and alkaline phosphatase levels were measured as indicators of oral inflammation (Nieminen et al. 1993) and alveolar bone resorption (Totan et al. 2006), respectively. Interestingly, only the test group showed a reduction in total protein level that seem to be compatible with the MGI and PD reductions, also confirming the findings of Nieminen et al. (1993). The anti-inflammatory property of essential oils was suggested by Sekino & Ramberg (2005) and confirmed by Sharma et al. (2008), who observed IL-2 and IFN-γ reductions after treatment with essential oils. Using a different protocol, a 45-min. full-mouth debridement with an ultrasonic instrument, associated with 0.5% pvp-iodine irrigation, in patients with chronic periodontitis, Zanatta et al. (2006) observed a reduction in trypsin activity as evaluated by the chair-side BANA test 3 months after treatment.

Alkaline phosphatase levels are higher in subjects with periodontal disease than in periodontally healthy patients (Totan et al. 2006). Based on this concept, Yoshie et al. (2007) identified alkaline phosphatase as an important marker to be utilized when monitoring responses to periodontal therapy. However, in our study population, the provided treatment did not change the salivary levels of this enzyme. Although our baseline values were higher (test: 62.30 ± 8.63; placebo 62.29 ± 10.20) than periodontal disease salivary values (34.38 ± 1.5 UI/l) reported by Totan et al. (2006), they did not meet the 75 UI/l cut-off value suggested by Kugahara et al. (2008) to distinguish between pregnant women with periodontitis and the others without periodontitis. Additional studies should be conducted with more severe periodontitis patients who present more evident alveolar bone resorption.

A key point for successful periodontal therapy is the patients' compliance. This fact was reinforced by the consensus report of the 6th European Workshop on Periodontology, which stated that, irrespective of treatment modality, accurate plaque control and patient adherence to prescribed treatments are fundamental to long-term clinical success (Sanz & Teughels 2008). In the present study, 24 patients from the placebo group and 23 from the test group completely adhered to the proposed protocol. Mongardini et al. (1999) cited a better compliance by periodontal patients undergoing one-stage full-mouth disinfection than those receiving quadrant-by-quadrant therapy. We believe that daily phone calls to remind patients about their appropriate self-chemical plaque control may have contributed to our good results. In addition, therapy was well tolerated as no inconvenient or undesirable side effects were reported.

In summary, the mouthrinse containing essential oils with the one-stage full-mouth disinfection provided additional clinical benefits, especially in reducing plaque and gingivitis. The inflammatory properties of essential oils anti- as well as the safety in long-term use were also reinforced by our salivary findings. The main microbial benefit was related to the protocol and not to the chemical agent as increases in *S. sanguinis* prevalence was observed in both groups. Together, these results indicate that full-mouth disinfection using an essential oil mouthrinse provides a benefit for treating gingivitis in patients with mild periodontal disease.

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Address:
Sheila Cavalca Cortelli
Rua Professor Nelson Freire Campelo
343 – Jardim Eulália
Taubaté
São Paulo
SP
12010-700
Brazil
E-mail: cavalcacortelli@uol.com.br

Clinical Relevance

Scientific rationale for the study: Full-mouth disinfection seems to be beneficial for improving oral health. We investigated whether mild periodontitis patients would benefit from using essential oils as antiseptic for full-mouth disinfection.

Principal findings: Our results corroborate those of reports stating that essential oils have anti-plaque and anti-gingivitis effects. The anti-inflammatory properties of these oils were reinforced by the observation of a reduction in total protein level. Essential oils did not alter basic salivary characteristics. Full-mouth disinfection did not reduce periodontopathogens, but it was accompanied by an increase in the prevalence of *S. sanguinis*.

Practical implications: These results indicate that full-mouth disinfection using an essential oil mouthrinse provides a benefit for treating gingivitis in patients with mild periodontal disease.