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Comparison of the effects of cetylpyridinium chloride with an essential oil mouth rinse on dental plaque and gingivitis – a six-month randomized controlled clinical trial

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Abstract

Objective: To compare the effects of an experimental mouth rinse containing 0.07% cetylpyridinium chloride (CPC) (Crest Pro-Health®) with those provided by a commercially available mouth rinse containing essential oils (EOs) (Listerine®) on dental plaque accumulation and prevention of gingivitis in an unsupervised 6-month randomized clinical trial.

Material and Methods: This double-blind, 6-month, parallel group, positively controlled study involved 151 subjects balanced and randomly assigned to either positive control (EO) or experimental (CPC) mouth rinse treatment groups. At baseline, subjects received a dental prophylaxis procedure and began unsupervised rinsing twice a day with 20 ml of their assigned mouthwash for 30 s after brushing their teeth for 1 min. Subjects were assessed for gingivitis and gingival bleeding by the Gingival index (GI) of Löe & Silness (1963) and plaque by the Silness & Löe (1964) Plaque index at baseline and after 3 and 6 months of rinsing. At 3 and 6 months, oral soft tissue health was assessed. Microbiological samples were also taken for community profiling by the DNA checkerboard method.

Results: Results show that after 3 and 6 months of rinsing, there were no significant differences ($p = 0.05$) between the experimental (CPC) and the positive control mouth rinse treatment groups for overall gingivitis status, gingival bleeding, and plaque accumulation. At 6 months, the covariant (baseline) adjusted mean GI and bleeding sites percentages for the CPC and the EO rinses were 0.52 and 0.53 and 8.7 and 9.3, respectively. Both mouth rinses were well tolerated by the subjects. Microbiological community profiles were similar for the two treatment groups. Statistically, a significant greater reduction in bleeding sites was observed for the CPC rinse *versus* the EO rinse.

Conclusion: The essential findings of this study indicated that there was no statistically significant difference in the anti-plaque and anti-gingivitis benefits between the experimental CPC mouth rinse and the positive control EO mouth rinse over a 6-month period.

Key words: cetylpyridinium chloride (CPC); dental plaque; essential oils; gingivitis; mouth rinse; prevention

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Conflict of interest and source of funding statement

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Studies among various populations in developing countries demonstrate that gingivitis results from the accumulation of extensive plaque and calculus deposits and is a common feature among adults (Baelum et al. 1986, 1988). In the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) of the United States Public Health Service, 50% of the adults were identified as having gingivitis (Oliver et al. 1998).

There is consensus that meticulous and complete toothbrushing once per day is sufficient to maintain oral health and to prevent caries and periodontal diseases (e.g. Lang et al. 1973, Attin et al. 2005). Various methods including the use of toothbrushes and inter-dental toothpicks are regularly recommended for mechanical plaque control. However, in industrialized countries, the average person appears to brush for <2 min. each time they clean their teeth (MacGregor & Rugg-Gunn 1979).

Most patients do not achieve effective plaque removal from interdental areas with toothbrushing (Cumming & Löe 1973). The effectiveness of plaque removal is, among other aspects, dependent on the dexterity and thoroughness of the individuals as well as their compliance (e.g. Frandsen 1986, Wilson 1987). Clinical studies including meticulous self-performed plaque control, combined with professional prophylaxis procedures three to six times per year, can, indeed, prevent the progression of periodontitis (Axelsson & Lindhe 1981; Axelsson et al. 1991, 2004). Such extensive and time-consuming efforts to obtain maximal results from mechanical cleaning have provided the basis for implementing preventive concepts but, at the same time, also suggest the need for developing adjunctive agents for chemical plaque control.

The classic experiments of Löe et al. (1965) demonstrated that the accumulation of microbial plaque for 3 weeks predictably resulted in the development of generalized gingivitis. Likewise, plaque removal reversed clinical inflammation to gingival health. A large number of studies have confirmed these findings both in humans and in experimental animals (e.g. Lindhe et al. 1975, Payne et al. 1975, Page & Schroeder 1976, Moore et al. 1982, Brecx et al. 1987, 1988). Oral hygiene procedures may also favourably influence the ecology of the microbial floral in both shallow and deeper pockets (Siegrist & Kornman 1982, Dahlén et al. 1992, Al-Yahfoufi et al. 1995).

Poor oral hygiene is associated with the development of gingivitis. However, the relationship between the individual oral hygiene level and the development of periodontitis is not clear, and only relatively few sites with persistent gingivitis may progress to periodontitis (Ånerud et al. 1979, Listgarten et al. 1985, Haffajee et al. 1988, Lindhe et al. 1989, Merchant et al. 2002). Microbiological studies have shown that the quantity of plaque accumulation was only weakly correlated with the prevalence of periodontitis (Haffajee et al. 1988, Lindhe et al. 1989), while studies have documented shifts in the microbial composition during the development of gingivitis and periodontitis (Theilade et al. 1966, Loesche & Syed 1978, Socransky et al. 1991).

If chemical agents are to prevent or reverse gingivitis, it is necessary that they be effective in modifying the microbiota by selective elimination of pathogens without a negative impact of the commensal microbiota (Mandel 1988). In addition, some chemical agents may also possess anti-inflammatory properties (Goodson et al. 2004).

Chlorhexidine digluconate (CHX) mouth rinses, sprays, and/or gels may today be considered to be a gold standard for oral chemical plaque control with antiseptics (Sekino et al. 2003, 2004, Charles et al. 2004, Quirynen et al. 2005, Southern et al. 2006).

Recently, an alcohol-free oral rinse product was developed for the prevention of plaque formation and the development of gingivitis. This contained the antimicrobial ingredient cetylpyridinium chloride (CPC) in a high bioactive mouthrinse matrix. CPC acts primarily by penetrating the bacterial cell membrane that causes leakage of cell components, disruption of bacterial metabolism, inhibition of cell growth, and finally cell death (Mankodi et al. 2005, Quirynen et al. 2005, Witt et al. 2005).

Essential oil (EO) mouth rinses appear to kill microorganisms by disrupting their cell walls and by inhibiting their enzyme activities (Kubert et al. 1993, Fine et al. 2001). They prevent bacteria from aggregating with Gram-positive pioneer species, slow bacterial multiplication, and extract endotoxins from Gram-negative pathogens. This may reduce the bacterial load, slow down the plaque maturation, and decrease the plaque mass and its pathogenicity (Fine 1988). EO mouthwashes seem to be capable of penetrating the plaque biofilms (Ouhayoun 2003).

The objective of the present study was to compare the clinical and microbiological effects of an experimental CPC mouth rinse with that of a commercially available and widely used EO mouth rinse in an unsupervised 6-month randomized clinical trial.

Material and Methods

The study was approved by the Ethical Committee of the Canton of Berne, Switzerland (KEK Project 175-04), and all participating subjects signed informed consent. The inclusion and exclusion criteria for the subjects for enrollment in the trial are presented in Table 1.

The trial was a randomized, double-blind, parallel-group, single-centre (Department of Periodontology and Fixed Prosthodontics, School of Dental Medicine, University of Berne) study. The subject number was determined after a power calculation for a β error of 0.2 using a data set from a previously performed study (Witt et al. 2005). Randomization was based on randomization tables and performed by an independent registrar. The allocation of the randomization and the enrolment of patients were performed by the same independent registrar at the start of treatment. During the treatment phase, the subjects used the investigational (test, CPC, Crest Pro-Health®, Procter & Gamble, OH, USA) or the commercially available (control) mouth rinse (EO, Listerine®, Johnson & Johnson) twice daily and for 6 months. Owing to the expectations of the subjects for this trial and in agreement with the recommendations of the Ethical Committee of the Canton of Berne, no negative placebo control mouth rinse was included in the study. At baseline, subjects received a dental prophylaxis procedure and began unsupervised rinsing twice a day with 20 ml of their assigned mouthwash for 30 s after brushing their teeth for

Table 1. Inclusion and exclusion criteria for the recruitment of the study population

Inclusion criteria
18–65 years, good general health
Written informed consent
Minimum of 18 natural teeth with measurable buccal and lingual surfaces
Mean GI score between 0.4 and 1.0 and a PlI score at least 0.6
Exclusion criteria
Widespread caries or chronic neglect
Antibiotic, anti-inflammatory or anticoagulant therapy or the use of oral rinses for 14 days before baseline
Medical conditions that may compromise the study results
Self-reported pregnancy or lactation
Orthodontic appliances or removable partial dentures
Advanced periodontal disease
History of hepatitis, diabetes
Rheumatic fever, heart murmur or other condition requiring prophylactic antibiocoverage

PlI, Plaque index; GI, Gingival index.

1 min. They were supplied with the mouth rinse, toothpaste, and a toothbrush every 6 weeks.

The clinical parameters were obtained at baseline, 3, and 6 months of treatment. Anti-plaque efficacy was assessed using the criteria of the Plaque index (PlI) (Silness & Löe 1964), while anti-gingivitis efficacy was determined by the criteria of the Gingival index (GI) (Löe & Silness 1963). Oral soft tissue examinations were conducted at each examination to monitor oral safety. All examiners were calibrated for reproducibility and blinded to the allocation of test or control mouth rinses. Bacterial profiles were analysed from 12 gingival bacterial samples per subject. These were obtained every third month. Microbiological samples were taken before all clinical examinations.

At the screening visit, medical history and demographic information was obtained and inclusion/exclusion criteria were reviewed. At the study baseline visit, the subject received oral soft tissue and oral hygiene assessments. Following this, the subjects received an oral prophylaxis and were randomly assigned to the experimental or the control group. The products were dispensed, and the subjects received verbal and written instructions on product usage. The mouth rinses were supplied in identical bottles. Subjects were instructed to rinse vigorously twice daily with 20 ml of rinse for 30 s after 1 min. of regular toothbrushing. Subjects returned every 6 weeks for product reissue and compliance checks.

Every sixth week, study compliance, a medical health history, and medication updates were obtained and adverse events were reviewed.

Microbiological processing

Subgingival plaque was obtained from the mesio-buccal surface of all molar sites in each patient. Following gentle removal of supragingival plaque with sterile curettes at each site, microbiologic samples were obtained by inserting sterile filter paper strips (Periopaper, Proflow Inc. Amityville, NY, USA) into the gingival sulci for 30 s. The samples were individually placed in Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). Within 30 min. after sampling, 0.1 ml 5 M NaOH was added to each tube. They were stored for the same period of time (3 months) before processing to avoid loss of microbiological information. The samples were analysed by the checkerboard DNA–DNA hybridization method (Socransky et al. 2004, Katsoulis et al. 2005).

A total of 40 bacterial strains were included in the analysis. Details of the method have been described elsewhere (Katsoulis et al. 2005, Gerber et al. 2006). In order to obtain a fully detailed account of the identified bacteria, the digitized information was analysed by a software program (ImageQuant, Amersham Pharmacia, Piscataway NJ, USA), allowing comparison of signals against standard lanes (10^5 or 10^6 cells) of known bacterial amounts. Signals were converted to absolute counts by comparison with these standards and studied as the proportion of sites defined as having $\geqslant 1.0 \times 10^5$ bacterial cells.

Statistical analysis

For all statistical analyses, the subject was the independent unit of observation. Descriptive statistics were used to define the study population using an intent-to-treat analysis. Analysis of covariance (ANCOVA) was used to study group and over time differences for the clinical parameters. The microbiological data were analysed by the Wilcoxon signed-rank test. Correlations between microbiological and clinical parameters were studied by Pearson's correlation and Spearman rank correlation.

Results

A total of 157 subjects fulfilled the entry criteria. Among these, 151 subjects (76 in the test group, and 75 in the control group) were finally enrolled and randomized into the study. In the test group, 56 women and 20 men, and in the control group, 55 women and 20 men volunteered to participated. In this study, 76% of the subjects were non-smokers (equally distributed in both groups). The mean age of the subjects in the test group was 39.5 years (SD $\pm$ 11.5, range 21–61) and 39.9 years (SD $\pm$ 11.1, range 21–62) ($p < 0.82$) in the control group.

At the 3-month examination, 11 subjects were lost to follow-up and adverse events in the test group, whereas 13 subjects were lost in the control group. Between the 3- and 6-month examinations, two more subjects were lost to follow-up and adverse events in the test group, while one subject was lost in the control group. Analysis of the data taking into consideration the drop-outs did not change the demographic distributions between the test and control groups. In the test group, the reasons for dropping out were: pain (four), stomatitis (one), dyspepsia (two), gingivitis (one) lost to follow-up (two), and one subject dropped due to protocol violation. In the control group, the reasons for dropping out were: pain (one), stomatitis (three), hyperesthesia (two), herpes simplex lesions (one), development of a periodontal abscess (one), dyspepsia (two), gingivitis (one), non-evaluable (one), and one lost to follow-up.

The attrition between baseline and 3 months (24 subjects) was largely due to self-reported adverse events.

Clinical results

The primary outcome variables were the GI values. The intent-to-treat analysis of

Table 2. Intent to treat comparison over time for Gingival index scores (Löe & Siness 1963) between the two rinsing groups. Descriptive statistics

Treatment group	*N*	Number of sites*	Baseline mean (SD)	Post-treatment mean (SD)	Change from baseline mean (SD)	Mean % difference *versus* baseline†
Month 3						
0.07% CPC rinse (Crest Pro-Health®)	76	142	0.80 (0.198)	0.55 (0.202)	− 0.26 (0.158)	32.5
Essential oil (Listerine®)	75	138	0.77 (0.242)	0.55 (0.233)	− 0.23 (0.179)	29.9
Month 6						
0.07% CPC rinse (Crest Pro-Health®)	76	138	0.80 (0.198)	0.56 (0.213)	− 0.25 (0.183)	31.3
Essential oil (Listerine®)	75	136	0.77 (0.242)	0.56 (0.236)	− 0.21 (0.210)	27.3

*Mean number of gradable sites per subject at baseline.
†The % benefit is calculated as 100 × (post-treatment mean − baseline mean)/(baseline mean).
CPC, cetylpyridinium chloride.

Table 3. Intend to treat comparisons as revealed by mean GI scores (Löe & Silness 1963) over time for all subjects. Analysis of covariance

Treatment group	*N*	Number of sites*	Baseline mean (SE)	Baseline *p*-value†	Adjusted mean (SE)	Treatment comparison	
						two-sided confidence interval‡	two-sided *p*-value
Month 3 (error variance = 0.0241)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	0.80 (0.023)	0.408	0.54 (0.018)	(− 0.06, 0.02)	0.456
Essential oil (Listerine®)	75	161 (1.1)	0.77 (0.028)		0.56 (0.018)		
Month 6 (error variance = 0.0318)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	0.80 (0.023)	0.408	0.55 (0.020)	(− 0.07, 0.02)	0.366
Essential oil (Listerine®)	75	161 (1.1)	0.77 (0.028)		0.57 (0.021)		

*Mean number of gradable sites per subject at baseline.
†Two-sided *p*-value from a two-sample *t*-test.
‡Two-sided 90% confidence interval on the adjusted mean difference.
CPC, cetylpyridinium chloride; GI, gingival index.

the mean GI is presented at the site level over time in Table 2, and the ANCOVA of the mean GI over time is presented in Table 3. After 3 or 6 months of mouthrinse application, there were no statistically significant differences in the mean GI values between the test and control groups. Neither was there a longitudinal effect of the mouth rinses between 3 and 6 months. When the data were analysed based on the proportion of sites with bleeding on probing (BOP) scores (GI = 2, 3), the results showed that at baseline, no statistically significant differences in bleeding scores existed between the two groups (test group: 8.7%, SD ± 9.8; control group: 9.3%, SD ± 8.3) (Fig. 1).

At the 3- and 6-month examinations, both groups demonstrated a statistically significant decrease in the percentages of bleeding sites (3 months: $p < 0.001$; 6 months: $p < 0.05$) compared with the baseline examination. However, at the 3-month examination, the reduction in the percentages of bleeding sites was greater in the test than in the control group, resulting in a statistically significant difference between the groups ($p < 0.001$). At the 6-month examination, this difference remained statistically significant ($p < 0.05$). Nevertheless, this statistically significant difference in the reduction of bleeding scores at 3 and 6 months was small (approximately 10%), thereby precluding any clinical significance.

For the analysis of supragingival plaque formation, the results of the descriptive statistics are presented over time in Table 4 and the ANCOVA over time in Table 5. There were no statistically significant differences between the test and control groups, neither by site nor by tooth group analysis. The analysis also failed to show longitudinal effects of the rinsing on the amount of supragingival plaque formation as revealed by the PII system, both at the 3- and 6-month examinations independent of the rinsing agent applied.

Tables 6 and 7 show an assessment of the bleeding tendencies for the two rinsing groups. The results of the analysis for this parameter represent the only aspect where statistically significant differences ($p < 0.05$) were observed between products after 3 and 6 months.

Microbiological results

At baseline, no correlation was found between the percentages of the sites BOP and the total bacterial load for any of the two groups (Fig. 2). Using 10% of sites with GI = 2, 3 as a cut-off for the presence of gingival inflammation in a subject, 30% presented with inflammation. Only three subjects had >30% of the sites yielding a score of GI = 2, 3. This lack of relationship between the two parameters did not change over time. Neither in the test nor in the control group did the total bacterial load change in the sulcular area over time based on the 40 species studied (Wilcoxon's signed-rank test). The total bacterial load and representation of individual bacteria are presented for the two rinsing groups (Figs 3 and 4). With the exception of *Capnocytophaga sputigena*, *Capnocytophaga showae, Neisseria mucosae*, and *Leptotrichia buccalis* (all at $p < 0.05$; Mann–Whitney *U*-test), no differences in other bacterial loads were found at baseline between the two groups.

At the 3- and 6-month examination, no group statistically significant differences in bacterial loads were identified in the sulcular area. Thus, neither mouth rinse reduced the bacterial load based on mean proportions. When dichotomized data (bacterial cut-off

at 1×10^5 organisms) were studied, no statistically significant differences were found over time either. For example at baseline, 49.0% of sites were positive for *Porphyromonas gingivalis*. At the 6-month examination, 43.9% were positive ($p = 0.24$) for *P. gingivalis*.

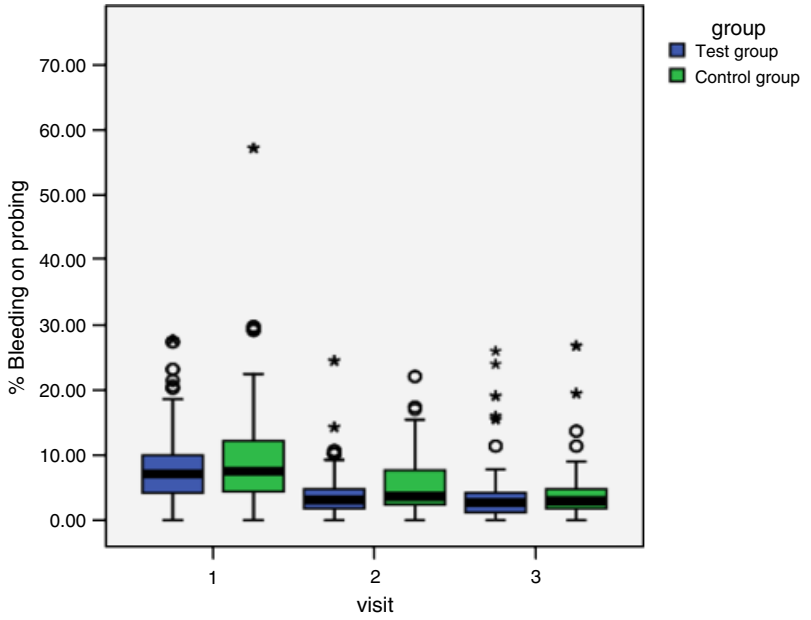

Fig. 1. Percentage of bleeding on probing for both test (cetylpyridinium chloride) and control (essential oil) groups at the various visits. Visit 1: baseline, visit 2: 3 months of rinsing, visit 3: 6 months of rinsing. *and o represent outliers.

Discussion

The present study has demonstrated that rinsing with an antiseptic mouth rinse applied daily significantly reduced the clinical evidence of gingival inflammation. This was documented both by a decrease of mean GI scores and mean percentages of sites with BOP between the baseline and the 3-month examinations. The results compare with those of previous studies on the preventive effects of first-generation antiseptic mouth rinses (Kornman 1986) both using the experimental gingivitis model (Siegrist et al. 1991) and 6-month trials of unsupervised rinsing (e.g. Grossman et al. 1989). In the latter study, the GI reductions at 3 months corresponded to approximately 14%, and bleeding sites were reduced by 15%, respectively, when compared with a placebo application. These values are closely related to the results of the present study and indicate a substantially reduced preventive effect of the EO compound when compared with rinses with chlorhexidine digluconate (e.g. Grossman et al. 1989) that had GI and bleeding site reductions amounting to more than double that encountered with

Table 4. Intent to treat comparisons over time as revealed by the Plaque index scores (Silness & Löe 1964) between the two rinsing groups. Descriptive statistics

Treatment group	*N*	Number of sites*	Baseline mean (SD)	Post-treatment mean (SD)	Change from baseline mean (SD)	Mean % difference *versus* baseline†
Month 3						
0.07% CPC rinse (Crest Pro-Health®)	76	142	0.45 (0.229)	0.34 (0.197)	− 0.11 (0.177)	24.4
Essential oil (Listerine®)	75	138	0.41 (0.232)	0.29 (0.192)	− 0.11 (0.170)	26.8
Month 6						
0.07% CPC rinse (Crest Pro-Health®)	76	137	0.45 (0.229)	0.31 (0.201)	− 0.14 (0.169)	31.1
Essential oil (Listerine®)	75	136	0.41 (0.232)	0.29 (0.192)	− 0.11 (0.183)	26.8

*Mean number of gradable sites per subject at baseline.
†The % benefit is calculated as 100 × (post-treatment mean − baseline mean)/(baseline mean).
CPC, cetylpyridinium chloride.

Table 5. Intent to treat comparisons as revealed by mean PII scores (Silness & Löe 1964) over time for all subjects. Analysis of covariance

Treatment group	*N*	Number of sites*	Baseline mean (SE)	Baseline *p*-value†	Adjusted mean (SE)	Treatment comparison	
						two-sided confidence interval‡	two-sided *p*-value
Month 3 (error variance = 0.0206)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	0.45 (0.026)	0.273	0.33 (0.017)	(− 0.01, 0.06)	0.268
Essential oil (Listerine®)	75	161 (1.1)	0.41 (0.027)		0.30 (0.017)		
Month 6 (error variance = 0.0215)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	0.45 (0.026)	0.273	0.30 (0.017)	(− 0.04, 0.03)	0.842
Essential oil (Listerine®)	75	161 (1.1)	0.41 (0.027)		0.30 (0.017)		

*Mean number of gradable sites per subject at baseline.
†Two-sided *p*-value from a two-sample *t*-test.
‡Two-sided 90% confidence interval on the adjusted mean difference.
CPC, cetylpyridinium chloride; PII, plaque index.

Table 6. Intent to treat comparisons as revealed by proportions of gingival bleeding over time. Descriptive statistics

Treatment group	*N*	Number of sites*	Baseline mean (SD)	Post-treatment baseline mean (SD)	Change from baseline mean (SD)	Mean % difference *versus* baseline†
Month 3						
0.07% CPC rinse (Crest Pro-Health®)	76	142	14.6 (12.67)	7.4 (6.22)	−7.1 (9.21)	48.6
Essential oil (Listerine®)	75	138	13.3 (10.99)	8.3 (8.05)	−5.0 (6.81)	37.6
Month 6						
0.07% CPC rinse (Crest Pro-Health®)	76	138	14.6 (12.67)	6.3 (6.17)	−8.3 (8.76)	56.8
Essential oil (Listerine®)	75	136	13.3 (10.99)	7.1 (7.95)	−6.2 (7.98)	46.6

*Mean number of gradable sites per subject at baseline.
†The % benefit is calculated as 100 × (post-treatment mean − baseline mean)/(baseline mean).
CPC, cetylpyridinium chloride.

Table 7. Intent to treat comparisons as revealed by proportions of gingival bleeding over time. Analysis of covariance

Treatment group	*N*	Number of sites*	Baseline mean (SE)	Baseline *p*-value†	Adjusted mean (SD)	Treatment comparison	
						two-sided confidence interval‡	two-sided *p*-value
Month 3 (error variance = 23.2748)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	14.6 (1.45)	0.515	7.1 (0.55)	(−2.75, −0.15)	0.027
Essential oil (Listerine®)	75	161 (1.1)	13.3 (1.27)		8.6 (0.56)		
Month 6 (error variance = 24.6758)							
0.07% CPC rinse (Crest Pro-Health®)	76	162 (1.1)	14.6 (1.45)	0.515	6.0 (0.57)	(−2.74, −0.06)	0.046
Essential oil (Listerine®)	75	161 (1.1)	13.3 (1.27)		7.4 (0.57)		

*Mean number of gradable sites per subject at baseline.
†Two-sided *p*-value from a two-sample *t*-test.
‡Two-sided 90% confidence interval on the adjusted mean difference.
CPC, cetylpyridinium chloride.

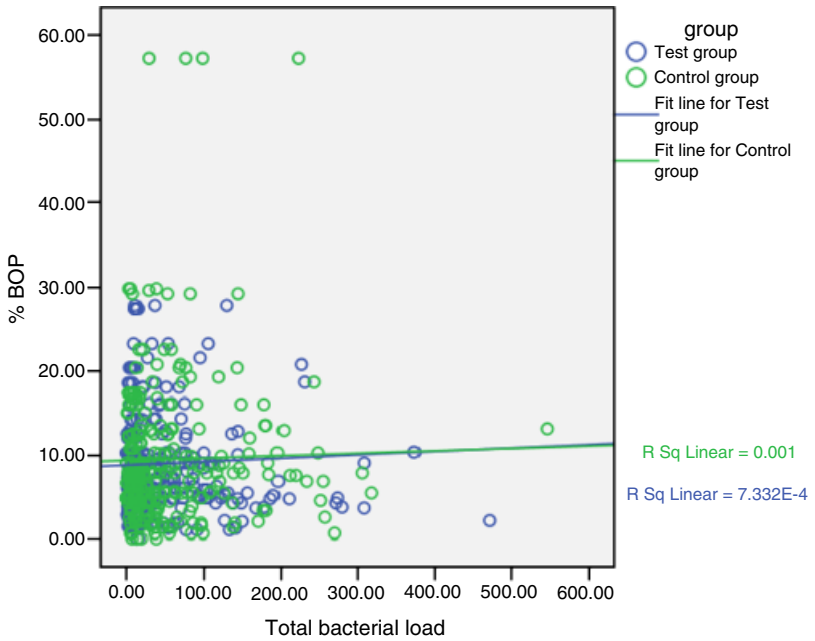

Fig. 2. Correlations between bleeding on probing (BOP) and total bacterial load for test (blue) and control (green) mouth rinses at the end of the study (after 6 months of mouth rinsing).

the EO mouth rinse. As in the study mentioned (Grossman et al. 1989), no further improvement was seen in the preventive clinical effects of the mouth rinses between 3 and 6 months of unsupervised application.

The essential finding of the present study was that the clinical effects of the test product containing 0.07% CPC did not differ significantly from those obtained with the traditionally marketed EO compound.

The plaque-inhibition property of the chlorhexidine (CHX) 0.2% solution and the non-alcohol-based 0.5% CPC was studied recently by Van Strydonck et al. (2005). The results showed no statistically significant differences in plaque accumulation between the two groups. The findings of the present study that oral rinses with an antiseptic agent did not have a clear impact on the sub-gingival microbiota disagree with those reported by others for chlorhexidine (Bollen et al. 1996, Santos et al. 2004, Quirynen et al. 2005). In those studies, the effects were demonstrated in patients with periodontitis, however. In one single study, a reduction of supragingival bacteria and bacteria in saliva has been reported applying chlorhexidine (Fine et al. 2001).

The reductions in % BOP following rinsing in the present study for both agents are consistent with those reported by Sekino & Ramberg (2005). Both site- and subject-based data analyses yielded similar results.

The hypothesis that such rinsing agents have an impact on the sub-gingi-

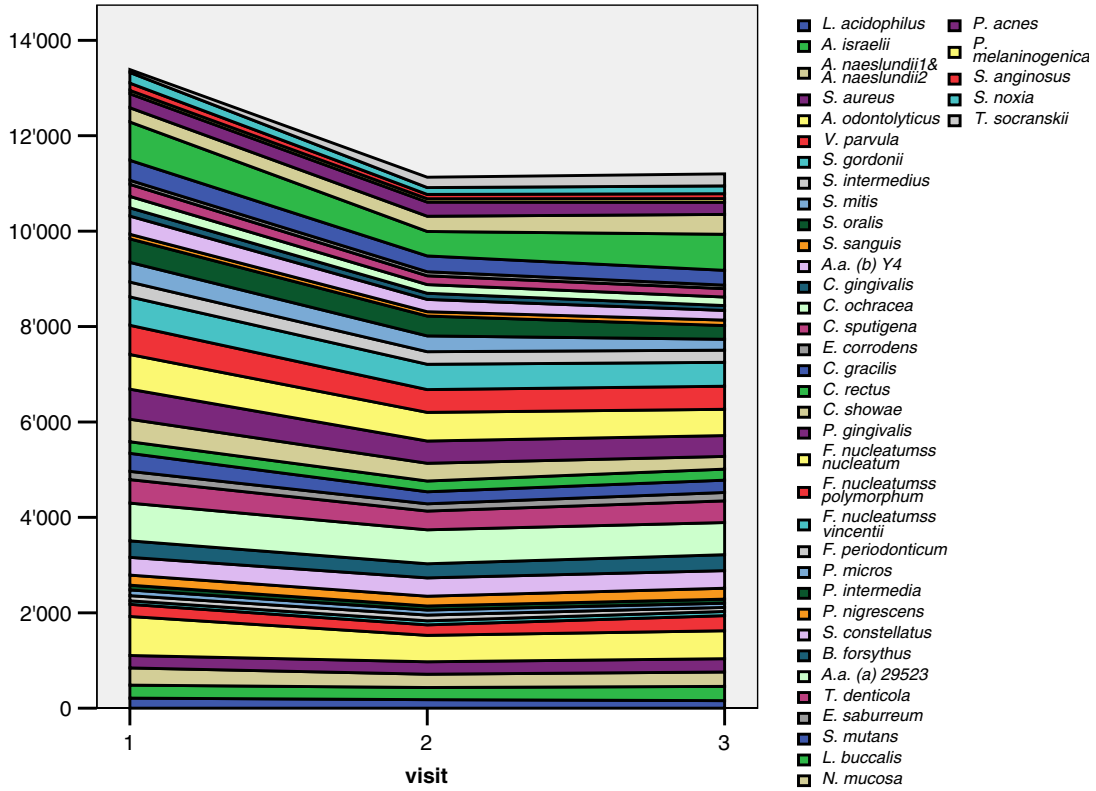

Fig. 3. Total bacterial load (40 periodontal organisms identified by checkerboard DNA–DNA hybridization) for the control (essential oil) group at the various visits. Visit 1: baseline, visit 2: 3 months of rinsing, visit 3: 6 months of rinsing.

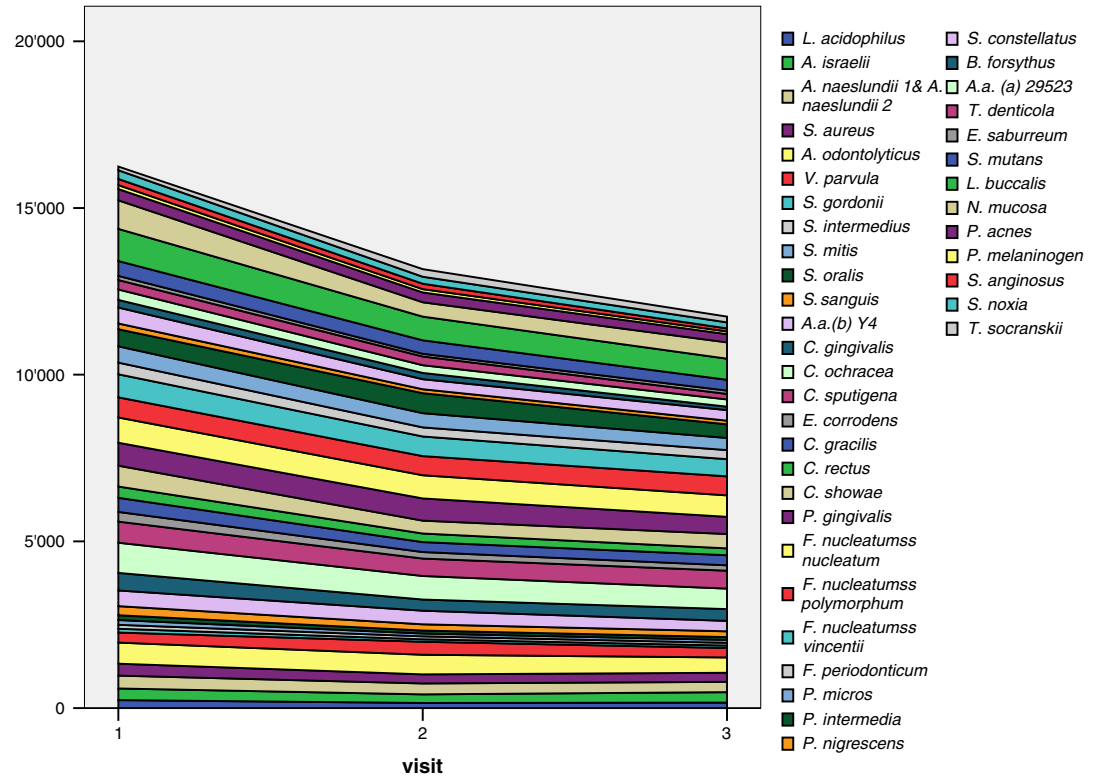

Fig. 4. Total bacterial load (40 periodontal organisms identified by checkerboard DNA–DNA hybridization) for the test (0.07% cetylpyridinium chloride) group at the various visits. Visit 1: baseline, visit 2: 3 months of rinsing, visit 3: 6 months of rinsing.

val microbiota was not validated in the present study. Further studies are needed to investigate these factors. In the present study, analysis of the data based on severity levels of gingivitis at baseline did not suggest that the rinses were more effective in subjects with greater plaque levels or more severe gingivitis.

The present study population consisted of relatively young adults and – as defined by the inclusion criteria – did not have evidence of periodontitis. Approximately 25% of the subjects participating had a smoking history. This rate appears to be substantially lower than that reported for Swiss adults (Ramseier 2005). The relatively low proportion of subjects with more than 10% of sites scoring GI = 2, 3 cannot be explained by the impact of smoking on the bleeding tendency. Within a few weeks at the beginning of the present study, a significant number of subjects (15.5%) were lost to follow-up. Because these drop-outs occurred at the beginning of the rinsing periods in both groups (test: 14,5% *versus* control: 17.3%), it may be speculated that the taste of the rinses did not appeal to these subjects, who are used to different flavouring agents applied in Europe as compared with the United States.

It was remarkable that approximately 50% of the sites harbored in the sulcular area *P. gingivalis* at the $\geqslant 1.10^5$ threshold level. As a result of rinsing, a trend towards lower bacterial loads that was similar in both groups was observed. However, these reductions did not reach statistical significance. This is of interest because the subjects also received a professional supragingival (sulcular) prophylaxis (polishing) both at baseline and at 6 months. Apparently, the combined effects of polishing and rinsing with an antiseptic agent failed to change the sulcular microbiota significantly. A Hawthorne effect in both groups may not be excluded.

In conclusion, the present study, applying either a test mouth rinse containing 0.07% CPC or EOs, but limiting toothbrushing time to 1 min. yielded no differences between the two agents in either affecting supragingival biofilm formation or gingival inflammation as expressed by the GI system. Because the study was statistically not set up for testing equality, it has to be realized that the ''no difference'' encountered for most of the parameters assessed, except for the BOP percentages, does not provide evidence for the clinical equality of the two products tested. Also, both agents affected the sulcular compartment in their microbial composition to a similar magnitude. However, there was a clinically small, but statistically significant benefit in reduction of bleeding sites after using the CPC rinse for 3 and 6 months compared with the EO rinse.

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Clinical Relevance

Scientific rationale for the study: A comparison of the clinical and microbiological effects of the daily rinsing with a high-bioavailability, alcohol-free 0.07% CPC mouth rinse with those of an alcohol-containing phenolic mouth rinse (Listerine®).
Principal findings: The present study compared the antigingivitis efficacy of a high-bioavailability, alcohol-free 0.07% CPC mouth rinse with that of a phenolic, alcohol – containing mouth rinse in a randomized-controlled clinical trial for 6 months. Both treatment regimens were found to be effective in reducing gingivitis (BOP) and slightly affected the sub-gingival microbiota, reducing the proportions of periodontal pathogens.
Practical implications: CPC represents an effective preventive antiseptic agent in the order of magnitude of a phenolic compound both in terms of reduction of BOP and periodontal pathogens.