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A randomized, controlled clinical trial on the clinical, microbiological, and staining effects of a novel 0.05% chlorhexidine/herbal extract and a 0.1% chlorhexidine mouthrinse adjunct to periodontal surgery

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Abstract

Background: Chlorhexidine (CHX) rinsing after periodontal surgery is common. We assessed the clinical and microbiological effects of two CHX concentrations following periodontal surgery.

Materials and methods: In a randomized, controlled clinical trial, 45 subjects were assigned to 4 weeks rinsing with a 0.05 CHX/herbal extract combination (test) or a 0.1% CHX solution. Clinical and staining effects were studied. Subgingival bacteria were assessed using the DNA–DNA checkerboard. Statistics included parametric and non-parametric tests ($p < 0001$ to declare significance at 80% power).

Results: At weeks 4 and 12, more staining was found in the control group ($p < 0.05$ and $p < 0.001$, respectively). A higher risk for staining was found in the control group (crude OR: 2.3:1, 95% CI: 1.3 to 4.4, $p < 0.01$). The absolute staining reduction in the test group was 21.1% (9 5% CI: 9.4–32.8%). Probing pocket depth (PPD) decreases were significant ($p < 0.001$) in both groups and similar ($p = 0.92$). No rinse group differences in changes of bacterial counts for any species were found between baseline and week 12.

Conclusions: The test CHX rinse resulted in less tooth staining. At the study endpoint, similar and high counts of periodontal pathogens were found.

Key words: chlorhexidine; gingivitis; herbal extract; microbiota; periodontal surgery; prevention; plaque; staining

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Conflict of interest and sources of funding statement

With regard to any process or product used to perform the present study, none of the authors have a conflict of interest.
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Aerobic and anaerobic bacteria in a complex biofilm are the primary aetiological factors of caries, gingivitis, periodontitis, and peri-implantitis (Carlen et al. 1996, Costerton et al. 1999, Rosan & Lamont 2000, Hall-Stoodley et al. 2004, Seki et al. 2006). Studies have shown that the bacterial colonization on tooth surfaces is a critical factor in the development of both gingivitis and periodontitis (i.e. Egelberg 1965, Socransky et al. 1998, Kroes et al. 1999, Socransky & Haffajee 2005, Haffajee et al. 2008). Once the biofilm is well established, it is difficult to eliminate the biofilm (Falagas et al. 2009, Souza et al. 2009, Verkaik et al. 2009). Adjunct antibacterial agents to control for the bacterial colonization of tooth surfaces have been studied extensively.

The scientific evidence on the efficacy of short-term CHX mouth rinsing with regard to the reduction of dental plaque and gingival inflammation is extensive (i.e. Flötra et al. 1972, Rindom-Schiott et al. 1976a, b, Axelsson & Lindhe 1987, Addy & Wade 1988, Persson et al. 1991, Albandar et al. 1994, Al-Tannir & Goodman 1994, Christie et al. 1998, Gunay et al. 1998, De Soete et al. 2001, Axelsson et al. 2004, Nomura et al. 2004, Arweiler et al. 2006). Rinsing with 0.12% CHX alone during prolonged periods, but without professional regular debridement does not appear to reduce subgingival bacterial counts in subjects with periodontitis (Persson et al. 2007). In comparison with CHX rinses, no other mouth rinses have shown superiority to control bacterial colonization or gingival inflammation (Moran et al. 1992, Jenkins et al. 1994, Renton-Harper et al. 1998, Pizzo et al. 2004).

Rinsing with CHX does not appear to exert long-term clinical side effects (Rushton 1977), and does not induce bacterial resistance (Jarvinen et al. 1995). The use of CHX in critical care units may reduce the risks for methicillin-resistant *Staphylococcus aureus* (MRSA) (Climo et al. 2009) infections. Effects against MRSA strains have also been demonstrated when CHX is used in the dental management of subjects in nursing home facilities (Hall 2003). In a randomized, controlled clinical trial on bone marrow transplant patients, prophylactic CHX rinses produced significant reductions in the incidence and severity of oral mucositis with concomitant reductions of oral *Streptococci* spp. and oral candida counts (Ferretti et al. 1988).

Rinsing with CHX has two primary side effects: Subjects may (I) experience a temporary loss of taste (Lang et al. 1988) and (II) develop staining on teeth (Johansen et al. 1975, Lang & Räber 1981), prosthetic appliances, and on the back of the tongue. Such a staining is tedious and usually requires professional assistance to be removed (Noiri et al. 2003). If a modified CHX solution could retain the same beneficial antibacterial effects, but not cause tooth staining, compliance would most likely improve.

Staining of teeth has been considered to be a non-serious adverse effect, which can also be the result of food products, wine (Berger et al. 2008), mouth rinses, or medications. The consumption of tea or coffee in combination with CHX rinsing can induce an increased risk for tooth staining (Leard & Addy 1997). Such increased risks of staining may, however, also occur in heavy tea and coffee drinkers without adjunct rinsing with CHX (Attia et al. 2009). In addition, the tooth staining effects of tobacco products is well known. The Lobene index (Lobene 1968) is a commonly used method to assess tooth staining in studies examining various oral hygiene methods (i.e. Axelsson & Lindhe 1987, García-Godoy & Ellacuria 2002, Van Strydonck et al. 2004, Terézhalmy et al. 2009). A modified version of the Lobene index has also been suggested (Lang & Räber 1981).

The Lobene index is similarly used for the assessment of tooth staining (Lobene 1968). Other modified indices to assess tooth staining have also been proposed (Lang & Räber 1981). It is highly unlikely that the index by Lang & Räber (1981) would have yielded different test results. The efficacy of a CHX mouth rinse solution to control for gingival inflammation originates from the same time as the establishment of the gingival and plaque indices (Davies et al. 1973, Löe 1973, Löe et al. 1976). The scientific literature on CHX as a mouth rinse to control for caries and gingivitis is extensive.

The literature on tooth staining and loss of taste perception following the application of CHX rinses is also extensive. Subjects who rinse with CHX solutions complain over non-serious adverse events that may occur in 30% of cases (McCoy et al. 2008). Others have shown that rinsing with 0.2% alcohol-free CHX for 1 week causes more irritation to oral mucosa, greater burning sensation, and increased altered taste perception compared with a placebo non-CHX rinse (Gürgan et al. 2006). Changes in the taste perception are of a short duration and easily reversible by subjects discontinuing the mouth rinse use and receiving dental prophylaxis (Lang et al. 1988, McCoy et al. 2008). The fact that rinsing with CHX as either a 0.2% or a 0.1% solution causes staining of teeth and, hence, may result in poor compliance has been discussed in several reports (i.e. Tilliss 1999, Charles et al. 2004, Cortellini et al. 2008).

The effects of CHX as an adjunct mouth rinse in combination with professional subgingival debridement have been studied extensively (i.e. Christie et al. 1998, De Soete et al. 2001). Studies of professional subgingival daily irrigation with 2% CHX solution in deep periodontal pockets in combination with debridement have, however, failed to demonstrate clinical benefits additional to the effects of debridement alone (Braatz et al. 1985). The clinical and microbiological data currently available on other applications of CHX such as the CHX chip treatment is limited, and the results are conflicting (Cosyn & Wyn 2006).

Following periodontal surgery, CHX rinsing is commonly prescribed (for review, see Heitz-Mayfield et al. 2002). Few studies have, however, assessed the antibacterial effects of CHX rinsing following surgical periodontal interventions (Sanz et al. 1989). Clinical results suggest that surgical periodontal therapy with adjunct CHX rinsing may eliminate or reduce counts of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Tannerella forsythia* (Shiloah et al. 1997). Studies on the clinical outcomes of periodontal surgical procedures with and without post-surgical rinsing with CHX have shown similar plaque control outcomes (Westfelt et al. 1983). Rinsing with CHX after periodontal surgery testing the development of staining of teeth between a 0.2% CHX solution with sodium metabisulphite and ascorbic acid, and a standard 0.2% CHX solution showed significantly less staining with the CHX including sodium metabisulphite and ascorbic acid than with a standard CHX solution (Cortellini et al. 2008).

The present study was designed as a randomized, double-masked, controlled clinical trial to assess the efficacy of a 0.05% CHX gluconate rinse with herbal extracts and sodium fluoride in comparison with that of a commonly used and commercially available 0.1% CHX gluconate rinse following periodontal surgery. We tested the null hypothesis that there are no differences in clinical outcomes with regard to: (i) probing pocket depth reduction, (ii) tooth staining side effects, and (iii) no effects on the subgingival microbiota during the first 12 weeks following open flap periodontal surgery.

Materials and Methods

Subjects

The ethics committee of the Canton of Berne, Switzerland, approved the pre-

sent study. All subjects signed the informed consent forms. The CONSORT guidelines were followed for clinical trials.

Clinical procedures

All patients had a clinical diagnosis of chronic periodontitis, and had been scheduled for surgical periodontal intervention by routine open flap surgery. None of the surgical procedures included the anterior teeth. No professional supragingival polishing or debridement was performed after surgery or before the end of the study. All subjects brushed their teeth with toothpaste containing sodium lauryl sulphate . The toothpaste neither contained CHX nor contained agents known to remove tooth staining. During surgery, root surfaces were debrided. The surgical wounds were rinsed with sterile salt solutions. Surgical flaps were repositioned using an interrupted inter-proximal suturing technique. The periodontists performing the surgical procedures were not time restricted by study protocol and could use hand instruments and ultrasonic equipment for debridement purposes as deemed appropriate.

Inclusion criteria

Diagnosis of chronic periodontitis and completion of initial cause-related periodontal therapy with a need for surgical periodontal intervention due to residual PPD $\geqslant 5$ mm at $\geqslant 3$ non-adjacent periodontal sites, and with the presence of bleeding on probing at re-evaluation.

Exclusion criteria

- Medical history of diabetes mellitus or cardiovascular disease;
- intake of systemic antibiotics during the preceding 6 months;
- currently on prescribed anti-inflammatory drug therapy;
- women known to be pregnant;
- changes in smoking habits within the last 6 months; and
- failure to comply with the rinsing regimen or scheduling of follow-up visits.

Allocation concealment

In this randomized, controlled clinical trial, subjects were randomly assigned to a test or a control group using a computer-generated assignment procedure (SPSS 16.0, SPSS Inc., Chicago, IL, USA).

Subjects received instructions on how to rinse twice daily with the solution during the first 4 weeks after periodontal surgery. All subjects received bottles with the rinse agent identified as either test solution ''B'' or ''C''. These bottles were otherwise exactly identical in terms of shape, colour, and material. Neither subject nor clinicians were informed about group allocation. The principal investigator who did not participate in treatment or data collection had access to a sealed envelope with allocation information, and had access to this information once data analysis was performed. Each bottle contained sufficient solution to last through the study rinse period of 4 weeks.

Clinical parameters

Staining of the six maxillary anterior teeth was assessed using the Lobene index (Lobene 1968). Evidence of tooth staining was assessed at baseline and at weeks 2, 4, and 12 after surgery. The intensity and extent of staining on the gingival crescent and body of the tooth, on the buccal surfaces of six maxillary anterior teeth were observed using a four-point scale: 0 = no visible staining, 1 = light staining, 2 = moderate staining, 3 = heavy staining.

Microbiological data were collected immediately before surgery, and at weeks 2, 4, and 12 after surgery. Subgingival bacterial samples were collected over time at the same four periodontal sites. Supragingival plaque was first removed, and bacterial samples were collected with sterile endodontic paper points (absorbent paper point size 55, Dentsply, Maillefer, Ballaigues, Switzerland). Samples were individually placed in labelled Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). The samples were stored at -20°C for 3–4 weeks and processed at the Oral Microbiology laboratory at the School of Dentistry, University of Berne, Switzerland. Before processing, 0.5 ml NaOH was added to the samples. Samples were analysed using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994). A total of 40 bacterial strains were included in the checkerboard panel. The reference strains used in the checkerboard are listed (Table 1). Whole genomic DNA probes and sample DNA precipitation were obtained (i.e. Socransky et al. 1994, 2004, Persson et al. 2008). In brief, bacterial DNA was extracted, concentrated on nylon membranes (Roche Diagnostics GmbH, Mannheim, Germany) and fixed by cross-linking using ultraviolet light (Stratalinker 1800, Stratagene, La Jolla, CA, USA). The membranes with fixed DNA were placed in a Miniplotter 45 (Immunetics, Cambridge, MA, USA). Signals were detected by chemifluorescence using the Storm Fluor-Imager (Storm 840, Amersham Biosciences, Piscataway, NJ, USA) with a set-up of 200 μm and 600 V. The digitized information was analysed using a software program (ImageQuant, Amersham Pharmacia, Piscataway, NJ, USA) allowing comparison of the density of 19 sample lanes against the two standard lanes (10^5 or 10^6 cells) and converted to absolute counts by comparisons with these standards (Socransky et al. 2004).

PPDs were assessed at the same sites from which bacterial samples had been sampled. This was performed immediately before periodontal surgery and at week 12.

The test and control mouth rinses

The test rinse (Parodentosan®, Tentan AG, Ramlinsburg, Switzerland) was based on a 0.05% CHX digluconate solution, and plant extracts (peppermint oil 0.1 g, tinctura myrrhae 1.9 mg, salviae aetheroleum 0.5 mg, sodium fluoride 0.11 g, zylitol 3 g, H_2O 77.8 g, glycerine 3.0 g, and alcohol 15% vol.). This mouth rinse was, in 2005, approved by Swissmedic (Bern, Switzerland), and is currently available in Swiss drug stores as an over-the-counter agent. The efficacy of this modified CHX formulation has not been tested previously. In the present study, the standard of care control mouth rinse was a 0.1% CHX solution (Plakout®, KerrHawe SA, Bioggio, Switzerland).

Statistical analysis

Independent *t*-tests were used for numerical data with normal distribution, whereas repeated Mann–Whitney *U*-tests were used for all microbiological data and for the analysis of staining

Table 1. Microbiological profile for the checkerboard DNA–DNA hybridization panel

Species	Type strain
Aggregatibacter actinomycetemcomitans (b)	ATCC 29523
Aggregatibacter actinomycetemcomitans (Y4)	ATCC 43718
Actinomyces israelii	ATCC 12102
Actinomyces naeslundii	ATCC 12104
Actinomyces odontolyticus	ATCC 17929
Campylobacter gracilis	ATCC 33236
Campylobacter rectus	ATCC 33238
Campylobacter showae	ATCC 51146
Capnocytophaga gingivalis	ATCC 33624
Capnocytophaga ochracea	ATCC 33596
Capnocytophaga sputigena	ATCC 33612
Eikenella corrodens	ATCC 23834
Eubacterium saburreum	ATCC 33 271
Fusobacterium nucleatum naviforme	ATCC 49256
Fusobacterium nucleatum nucleatum	ATCC 25586
Fusobacterium nucleatum polymorphum	ATCC 10953
Fusobacterium periodonticum	ATCC 33693
Lactobacillus acidophilus	ATCC 11975
Leptothrichia buccalis	ATCC 14201
Neisseria mucosa	ATCC 19696
Parvimonas micra	ATCC 33270
Porphyromonas gingivalis	ATCC 33277
Prevotella intermedia	ATCC 25611
Prevotella melaninogenica	ATCC 25845
Prevotella nigrescens	ATCC 33563
Proprionybacterium acnes	ATCC 11827
Selenomonas noxia	ATCC 43541
Staphylococcus aureus	DSMZ 20231
Streptococcus anginosus	ATCC 33397
Streptococcus constellatus	ATCC 278233
Streptococcus gordonii	ATCC 10558
Streptococcus intermedius	ATCC 27335
Streptococcus mitis	ATCC 49456
Streptococcus mutans	ATCC 25175
Streptococcus oralis	ATCC 35037
Streptococcus sanguinis	ATCC 10556
Tannerella forsythia	ATCC 43037
Treponema denticola	DSMZ 14222
Treponema socranskii	D40D82
Veillonella parvula	ATCC 10790

ATCC, American Type Culture Collection; D, sample from Forsyth Institute, Boston, MA; GUH, Ghent University Hospital Collection, Ghent, Belgium; DSMZ, the German Resource Center for Biological Materials, Braunschweig, Germany.

scores In the between-group analysis, the Wilcoxon signed rank test was used to assess within-group differences for non-parametric data. The binary logistic regression analysis was included to assess explanatory factors. Crude Mantel–Haenszel odds ratio statistics were used to assess the number of subjects needed to treat (NNT). The effects on rinsing were defined as the primary outcome measure. We assumed a 40% difference in changes of staining (Lobene index) between test and control groups. Thus, if 20 subjects were included in each group, the statistical power would be 85%. The SPSS 17.0 statistical software package for MAC was used for the analysis (SPSS Inc., Chicago, IL, USA).

Results

Through the screening process, three subjects did not meet the inclusion criteria (Fig. 1). The enrolment process resulted in a study population of 45 subjects, with 23 subjects in the test rinse group and 22 subjects in the control rinse group. In the study, 45 subjects (24 women) with a mean age of 53.8 years (SD ± 12.8) were enrolled. None of these subjects were lost to follow-up.

PPD

At baseline and at the sites sampled and treated surgically, a PPD = 4 mm was found in 13.1% of sites, PPD = 5 mm was observed in 33.7%, PPD = 6 mm was observed in 34.3%, and PPD > 6 mm was noted in 32.0% of sites. At week 12, the distributions of PPDs at the same sites were as follows: PPD < 4 mm in 49.7% of sites, PPD = 4–5 mm in 31.8%, PPD = 6 mm in 9.6%, and PPD > 6 mm in 9.0% of the sites. Thus, at baseline, the mean PPD values at microbiologically sampled sites in the test and control groups were 6.3 mm (SD ± 1.6 mm) and 5.8 mm (SD ± 1.4 mm), respectively. The baseline PPD values were significantly higher in the test group [mean difference: 0.5 mm, SE difference: ± 0.3 mm, 95% CI: 0.1–1.0 mm, $p < 0.02$ (equal variance not assumed)].

The decreases in PPD values between baseline and week 12 were statistically significant in both groups ($p < 0.001$). Statistical analysis failed to show within-site changes in PPD values between baseline and week 12 by group assignment (mean group difference: 0.1 mm, SE difference: ± 0.3 mm, $p = 0.88$). Statistical analysis also failed to show between-group differences in changes of PPD ($p = 0.92$).

Tooth staining

The baseline, week-4, and week-12 distributions of Lobene tooth staining scores in both groups are illustrated (Fig. 2). The proportional distributions of the Lobene index scores are also presented (Table 2). At baseline, the staining scores were significantly higher in the test group than in the control group ($p < 0.001$). Thus, at baseline, a Lobene score ⩾1 was identified at 55.6% in the test group and in 24.7% in the control group. At weeks 2 and 4, statistical analysis failed to demonstrate study group differences in the extent of staining ($p = 0.62$ and $p = 0.23$, respectively). At the end of week 4, the extent of surfaces with evidence of tooth staining (Lobene score ⩾1) was 63.6% in the test rinse group and 74.0% in the control rinse group. At week 12, the extent of tooth surfaces with staining was 63.0% or 7.4% higher than that at baseline in the test rinse group, and 61.5% or 36.8% higher than that at baseline in the control rinse group. The differences in changes of staining scores are illustrated (Fig. 3). The percentage values are based on respective group merged scores.

Analysis by Kruskal–Wallis ANOVA for data within the test rinse group failed to demonstrate changes in the extent of

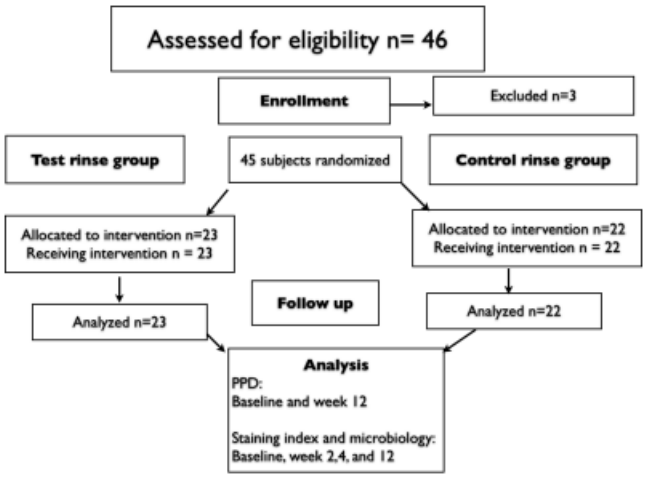

Fig. 1. A CONSORT E flow chart of the enrolment, allocation, follow-up, and analysis.

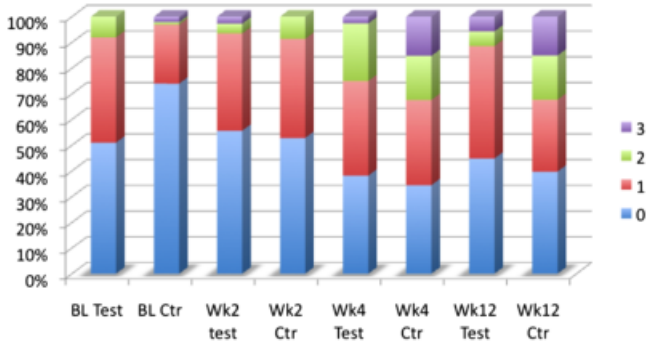

Fig. 2. The distribution of Lobene staining scores in the test and control groups between baseline and week 12.

staining ($p = 0.08$). Data analysis by repeat Mann–Whitney *U*-tests for results in the test group also failed to demonstrate significant differences in tooth staining between baseline and week 2 ($p < 0.06$), between baseline and week 4 ($p = 0.10$), or between baseline and week 12 ($p = 0.14$).

Analysis by Kruskal–Wallis ANOVA for data within the control rinse group demonstrated significant changes in the extent of staining ($p = 0.001$). Data analysis by repeat Mann–Whitney *U*-tests confirmed these results, with greater extent of tooth staining at week 2 ($p < 0.001$) than at week 4 ($p < 0.001$), and at week 12 ($p < 0.001$) when compared with baseline tooth staining scores.

The changes of staining between baseline and week 12 demonstrated a greater increase in staining in the control rinse group than in the test rinse group ($p < 0.001$). Pair-wise analysis (Wilcoxon signed rank test) failed to demonstrate a difference in the change of staining between baseline and week 12 in the test rinse group ($p = 0.12$). The statistical analysis identified significantly more staining at week 12 in the control rinse group ($p < 0.001$).

Combining the Lobene scores of 0 and 1 into one category and the Lobene staining scores of 2 and 3 in another category allowed risk assessment of staining between the two different CHX rinse agents. Analysis by Mantel–Haenszel common odds (crude ratios) demonstrated that tooth staining was more likely to occur in the control rinse group at week 12 (odds ratio: 2.3:1, 95% CI: 1.3–4.4, $p < 0.01$). Thus, the reduction of the absolute risk for the novel 0.05% CHX mouth rinse to result in tooth staining was 21.1% (95% CI: 9.4–32.8%). Thus, the NNT with the test mouth rinse to prevent staining would be five subjects (95% CI: 3.0–10.6%). Neither subjects in the test nor in the control rinse group complained about tooth staining at any time point of the study.

Subgingival microbiota

The proportional distribution of selected bacteria at baseline, week 4, and week 12 are presented by rinsing group assignment (Table 3). At baseline, statistical analysis failed to demonstrate differences in the bacterial counts for the 40 species studied. Statistical analysis also failed to demonstrate group differences in the sum of the total bacterial load. At week 2, significantly higher counts of *Capnocytophaga ochracea and Capnocytophaga sputigena* were found in the control rinse group ($p < 0.001$). Trends of higher bacterial counts in the control group were also found for *Streptococcus intermedius* ($p < 0.007$) and *Neisseria mucosa* ($p = 0.012$). At weeks 4 and 12, statistical analysis failed to demonstrate group differences in individual bacterial counts or for the sum of bacterial load of bacterial species assessed (*p*-values varying between 0.05 for *Streptococcus sanguinis* and 0.98 for *Lactobacillus acidophilus*).

The changes in bacterial counts between baseline and week 12 within each site sampled were assessed. Statistical analysis failed to demonstrate that the changes in bacterial counts between baseline and week 12 differed by rinse groups for any of the bacterial species studied. The changes in counts of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia* in the two study groups are presented in a box plot diagram (Fig. 4).

Analysis by binary logistic regression (Wald) to assess what changes were explanatory to the outcome for the changes in the Lobene index scores showed that only the bacterial counts of *Actinomyces israelii* were included in the model analysis.

Microbiological changes in the test rinse group

Analysis by Mann–Whitney *U*-tests demonstrated that between pre-treatment and 2 weeks after surgery (during active rinsing), a significant decrease in bacterial counts was found for the following species ($p < 0.001$): *Campylobacter rectus*, *N. mucosa*, *Treponema denticola*, and *T. forsythia*. Trends of deceases in bacterial counts were found for *P. gingivalis*, *Campylobacter showae*, and *Treponema socranskii* ($p < 0.01$). Statistical analysis failed to demonstrate differences in bacterial counts between baseline and weeks 4

Table 2. Proportional distributions (%) of Lobene staining index scores at baseline and at weeks 2, 4, and 12 after open flap debridement surgery.

Time	Lobene score	Test rinse Parodentosan®	Control rinse Plakout®
Baseline	0	44.4	75.3
	1	45.6	20.8
	2	8.9	1.3
	3	1.1	2.6
Week 2	0	39.0	36.6
	1	31.7	26.8
	2	28.0	28.2
	3	1.2	8.5
Week 4	0	36.4	26.0
	1	32.7	32.0
	2	27.3	26.0
	3	3.6	16.0
Week 12	0	37.0	38.5
	1	49.3	33.8
	2	6.8	20.0
	3	6.8	7.7

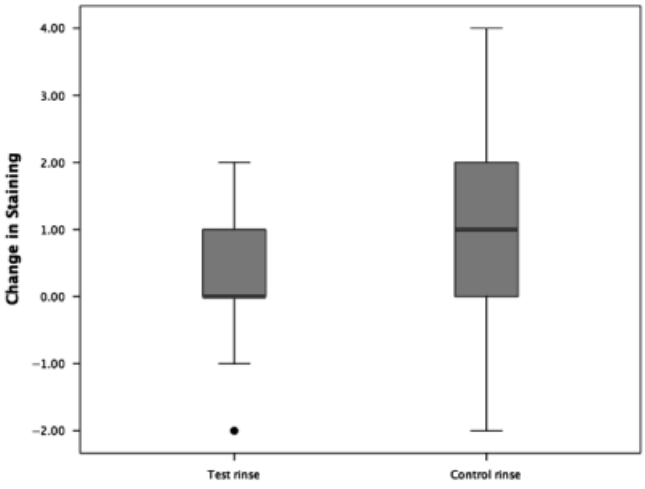

Fig. 3. Box plot diagram illustrating the changes in Lobene staining index scores between baseline and week 12. A positive score represents an increase in staining (• , outliers).

and 12 in the test rinse group. Trends of differences were found for several species including *P. gingivalis* ($p = 0.01$), *T. forsythia* ($p = 0.05$), and *T. denticola* ($p < 0.01$). Within-site analysis of subgingival bacterial changes between baseline and week 12 (pair-wise test) identified that only the subgingival counts of *P. gingivalis* had decreased ($p < 0.001$).

Microbiological changes in the control rinse group

Analysis by Mann–Whitney *U*-tests demonstrated that between pre-treatment and 2 weeks after periodontal surgery and during continuing rinsing with the control solution, decreases in the counts of the following bacterial species were found at the $p < 0.001$ level: *A. israelii*, *Actinomyces naeslundii*, *C. rectus*, *C. showae*, *Fusobacterium nucleatum naviforme*, *Fusobacterium nucleatum nucleateum*, *Fusobacterium nucleatum polymorphum*, *Prevotella intermedia*, *Prevotella nigrescens*, *P. gingivalis*, *T. forsythia*, *T. denticola*, and *T. socranskii*. In addition, the sum of the bacterial load of species studied was lower at week 2 than at baseline ($p < 0.001$). At week 4, the following bacterial species were found with decreased bacterial counts in the control group compared with baseline ($p < 0.001$): *C. showae*, *C. rectus*, *P. gingivalis*, *T. forsythia*, and *T. denticola*. The sum of the bacterial load for species studied demonstrated a trend towards lower counts at week 4 ($p = 0.007$). In comparison with baseline, only *P. gingivalis* and *T. forsythia* were identified at lower counts at week 12 ($p < 0.001$). Trends of lower counts ($p < 0.01$) were found for *A. naeslundii* and *C. rectus*. Within-site analysis of subgingival bacterial changes between baseline and week 12 (pair-wise test) identified that the subgingival counts of *A. actinomycetemcomitans* (Y4), *C. rectus*, *P. gingivalis*, and *T. forsythia* had decreased ($p < 0.001$).

Discussion

In the present study, the data demonstrated that rinsing with a 0.05% CHX solution with added herbal extracts and sodium fluoride resulted in a decrease of tooth staining both at week 4 and at week 12. The study revealed that at week 12, the likelihood of clinically disturbing staining score (Lobene index scores $\geqslant 2$) was higher in the control rinse group. The NNT analysis identified that the test rinse resulted in a 21% reduction of tooth staining in comparison with the 0.1% CHX control rinse. This reduction in tooth staining in comparison with the usually applied 0.1% CHX solutions is clinically relevant.

Essential oils of several plants are widely used in ethno-medicine for their antimicrobial and anti-inflammatory properties. For example, extracts from salvia might be valuable in the development of new antimicrobial agents (Lee et al. 2007). Few studies have assessed the effects of essential oils on periodontal conditions and bacteria associated with periodontitis. Data suggest that essential oil preparations inhibit the growth of bacteria associated with periodontitis (Gursoy et al. 2009). Studies have also demonstrated that plant extracts may inhibit oral microbial growth similar to what is known for CHX solutions (Feres et al. 2005). The present study supported this evidence in that the combination of a low-concentration CHX gluconate with herbal extracts possessed similar capacity to control recolonization of subgingival bacterial as rinsing with a 0.1% CHX solution.

Although the group allocation was performed by computer-based randomization, baseline differences in both PPD and Lobene staining index scores were found and with higher scores in the test rinse group. Therefore, it is interesting to note that in spite of the baseline scores in the test rinse group, a change to significantly lower staining scores

Table 3. The distribution of select bacteria at baseline, week 4, and week 12 in test and control rinse groups, and presented as the proportion of sites with bacterial presence at a cut-off level $\geqslant 1.0 \times 10^5$ bacterial cells.

Select bacterial species $\geqslant 1.0 \times 10^5$ cells	Test rinse group			Control rinse group		
	Baseline	Week 4	Week 12	Baseline	Week 4	Week 12
Actinomyces israelii	18	18	21	24	10	14
Actinomyces naeslundii	27	17	21	35	19	18
Lactobacillus acidophilus	40	36	38	52	40	46
Streptococcus gordonii	61	30	30	33	38	46
Streptococcus mutans	31	20	34	31	19	40
Staphylococcus aureus	33	31	31	36	26	33
Aggregatibacter actinomycetemcomitans Y4	31	28	22	58	41	29
Prevotella intermedia	39	41	38	40	34	40
Parvimonas micra	47	32	37	55	28	31
Porphyromonas gingivalis	47	26	31	55	28	31
Tannerella forsythia	62	36	46	60	28	38
Treponema denticola	55	31	42	43	34	47

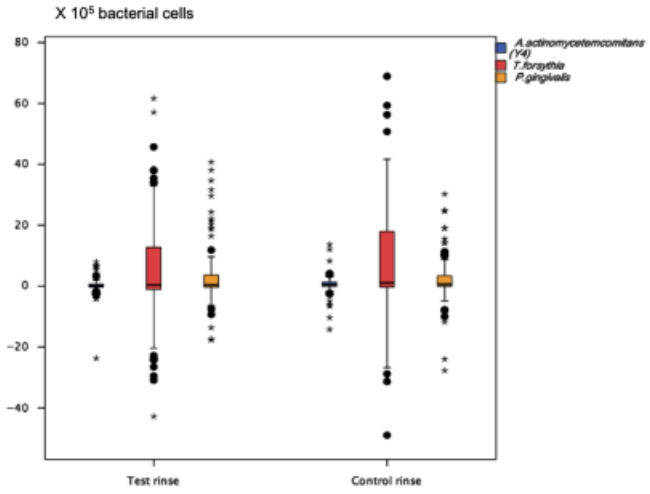

Fig. 4. Box plot diagram illustrating the changes in counts ($\times 10^5$ cells) of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia*. Positive changes suggest an increase in bacterial counts between baseline and week 12 (• , outliers and *extreme outlier values) (values on the *Y*-axis: $\times 10^5$ bacterial cells)

was found at the end of the study. This suggests that the 0.05% CHX solution with herbal extracts and sodium fluoride may have the ability to enhance stain removal as an adjunct to tooth brushing.

The baseline difference in PPD in the test and control groups may, in part, be explained by probing errors. The mean difference in PPD between groups observed (0.5 mm, 95% CI: 0.1–1.0 mm) is consistent with the margin of error in PPD assessments with non-forced controlled probing (Osborn et al. 1992).

The reduction in PPD at the sites of periodontal surgery was anticipated. Because of the fact that the primary outcome assessment was the effects on staining using the mouth rinse after surgery, the surgical procedures also included the use of hand and/or ultrasonic instruments as preferred by the clinician. The clinician was also allowed to use the suturing technique of his/her choice. The reduction of PPD (mean reduction of 2 mm in both groups) encountered in the present study was consistent with that reported from other studies of periodontal therapy (Westfelt et al. 1983, Zybutz et al. 2000, Kim et al. 2007, Fickl et al. 2009). Nevertheless, a rather high proportion (approximately 20%) of sites with PPD $\geqslant$6 mm were still present at week 12. This may partially explain why the changes in the proportion of sites with remaining bacteria associated with periodontitis were only marginal in both rinsing groups. It should also be realized that the effect of antiseptics in residual pockets may be limited due to the subgingival ecosystem favouring the colonization with gram-negative anaerobic bacteria (Wennström et al. 1987, Persson et al. 2007). From a clinical periodontal perspective, the test rinse CHX solution is equivocal to rinsing with the standard of care 0.1% CHX gluconate mouth rinse solution. The data do not suggest that rinsing with a 0.1% CHX solution or a 0.05% CHX with herbal extracts impact on PPD changes following surgery. In order to address that question a thord study arm would have been necessary. This new group should have been periodontal surgery but not using the post-surgical adjunct rinsing with CHX. Owing to the fact that rinsing with an antibacterial mouth rinse after periodontal surgery was considered to be the standard of care by the clinicians and by the ethics committee, we followed the Helsinki declaration (Edinburgh amendment) (Carlson et al. 2004) testing the new 0.05% CHX rinse with herbal extracts against the standard of care post-surgical management. Although a statistically significant difference in baseline PPD was observed, the change (mean decrease) in PPD was similar and independent of rinse group allocations.

The evidence on the efficacy of bacterial control following periodontal surgery with or without the adjunct use of mouth rinses is limited. In the present study, we demonstrated a decrease of bacterial counts between baseline and week 4. The findings from the present study that rinsing with CHX resulted in a temporary reduction of bacteria in periodontal pockets are consistent with findings by others (Newman et al. 1989, Tuan et al. 2000, Levy et al. 2002, Ioannou et al. 2009).

In order to obtain optimal periodontal wound healing, it would important to control for bacterial at a longer period than 4 weeks. This is a major challenge for the clinician as the oral cavity harbours a considerable variety of bacteria and in high concentrations also under healthy normal conditions (Paster et al. 2006).

The extent of bacterial recolonization between weeks 4 and 12 was, however, unexpected, but consistent with findings of other studies. (Quirynen et al. 2005, Fürst et al. 2007). It is likely that the protocol of post-surgical rinsing for 4 weeks was insufficient to control bacterial recolonization during the wound-healing period. Rinsing with either a 0.1% CHX solution or a 0.05% CHX solution may also be suboptimal con-

centrations for the prevention of biofilm formation (Lang & Ramseier-Grossmann 1981, Lang et al. 1982). Subjects may also be required to rinse for periods longer than 4 weeks after periodontal surgery. The changes in bacterial counts after periodontal surgery were similar between the two groups. Furthermore, a significant variation in changes was identified in both groups. Thus, it appears that the surgical treatment with adjunct rinsing with CHX does not change subgingival bacterial presence as identified in the present study. This is consistent with the perception that it is almost impossible to eliminate a biofilm through mechanical means once the biofilm has been established (Falagas et al. 2009, Souza et al. 2009, Verkaik et al. 2009). Genetic predisposition may, furthermore, control bacterial colonization patterns (Papapanou et al. 2009).

As a result of the significant differences in tooth staining, but with limited differences in subgingival bacterial colonization, the test rinse (Parodentosan® with sodium fluoride) may confer a benefit in comparison with the standard of care 0.1% CHX solution in the long-term application of chemical plaque control in patients with insufficiently controlled oral hygiene.

In conclusion:

(I) We rejected the null hypothesis of no difference in non-adverse staining side effect in that the test rinse (0.05% CHX/herbal extracts/sodium fluoride) resulted in less tooth staining than occurring after rinsing with conventional 0.1% CHX solutions.

(II) We accepted the null hypothesis of no difference in PPD change as a result of periodontal surgery and rinsing with either the test 0.05% CHX rinse or the control 0.1% CHX rinse solution.

(III) We accepted the null hypothesis of no difference in bacterial colonization pattern following periodontal surgery as an effect of adjunct rinsing with either the 0.05% or the 0.1% CHX solution.

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Clinical Relevance

Scientific rationale for the study: Rinsing with CHX after periodontal surgery is common practice, but may cause tooth staining. The information on bacterial reduction following periodontal surgery is limited.

Principal findings: The study showed that rinsing with a 0.05% CHX solution with sodium fluoride and herbal extracts or rinsing with a 0.1% CHX do not differ in clinical outcomes as assessed by probing depth reduction and bacterial counts through the first 12 weeks after open flap debridement surgery. Rinsing with the 0.05% CHX solution causes less tooth staining.

Clinical implications: Rinsing with a 0.05% CHX/herbal extract solution reduces the risk for non-adverse tooth staining following surgery by 21% compared with rinsing with a standard of care 0.1% CHX solution.