Clinical and Microbiological Effects of Adjunctive, Locally Delivered Chlorhexidine on Patients with Chronic Periodontitis

Sakellari Dimitra[1], Loannidis Loannis[1], Antoniadou Malama[1], Slini Theodora[2] and Konstantinidis Antonis [1]
[1]Department of Preventive Dentistry, Periodontology and Implant Biology, and the [2]Department of Mechanical Engineering, Aristotle University of Thessaloniki, Greece

Abstract

Objective: The impact of a locally delivered chlorhexidine chip (Periochip®) on clinical and microbiological parameters of chronic periodontitis requires further documentation. The aim of the present study was to investigate the effects of the chip as an adjunct to mechanical treatment of chronic periodontitis. **Methods:** Fifty patients with chronic periodontitis were randomized into two groups. The test group (n = 25) received scaling and root planing and adjunctive Periochip® in four pockets. The control group (n = 25) received scaling and root planing only. Clinical indices (probing depth, probing attachment level, bleeding on probing) were assessed at baseline, three and six months. Subgingival samples were analyzed at baseline, three weeks, three and six months after treatment for levels of eight bacterial species using "checkerboard" DNA-DNA hybridization. **Results:** The targeted difference of probing depth of 2 mm between groups was not observed. Both treatments resulted in improvement of clinical indices and non-statistically significant differences were observed between the two groups at any time point, with the exception of bleeding on probing at three months (ANOVA, $p < 0.05$). No major differences were observed concerning levels of important periodontal pathogens (Mann-Whitney test, $p \leq 0.05$). **Conclusions:** In this small, six-month, phase 4 trial, no differences in mean probing depth reduction or "red-complex" periodontal pathogens were detected for patients with chronic periodontitis treated with adjunctive chlorhexidine chip (single administration) as compared to patients treated with scaling and root planing alone.

Key words: randomized controlled clinical trial, chronic periodontitis, chlorhexidine chip

Introduction

The incorporation of chlorhexidine (CHX) in controlled-delivery devices for subgingival application is based on its well established efficiency as an oral antiseptic (Addy and Moran, 1997; McDonnell and Russell, 1999; Baehni and Takeuchi, 2003). Results from studies evaluating a chlorhexidine chip (Periochip®) as an adjunct for treatment of chronic periodontitis have been conflicting. Data from multi-centered studies have shown additional benefits from CHX chips concerning clinical parameters of periodontal disease (Soskolne *et al.*, 1997; Jeffcoat *et al.*, 1998). In these studies, additional pocket reductions of 0.46 and 0.30 mm respectively were reported when the chip was applied in combination with mechanical treatment compared to scaling and root planing alone (Soskolne *et al.*, 1997; Jeffcoat *et al.*, 1998). Findings from other, smaller-scale studies have been contradictory. Furthermore, the effects of Periochip® on the subgingival microbiota appear to require more extensive documentation (Azmak *et al.*, 2002; Daneshmand *et al.*, 2002; Grisi *et al.*, 2002). Although the adjunctive effects of the chip have been shown in multicentered studies, in daily practice it is useful to know the upper limits of our clinical expectations and the treatment phase when they can be best accomplished. Therefore, information adding to current knowledge about the impact of the CHX chip at various phases of periodontal treatment can assist clinicians in estimating the cost benefit and decision-making in daily practice.

The aim of the present study was to evaluate the effects of adjunctive Periochip® on clinical and microbiological parameters in patients with chronic periodontitis at the initial treatment phase.

Correspondence to: Dimitra Sakellari, 88 Mitropoleos Str. Thessaloniki, 54622, Greece. e-mail: dimisak@med.auth.gr

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Materials and methods

Experimental design

Fifty-six subjects, patients of the Clinic of the Department of Periodontology and Implant Biology, Dental School, Aristotle University of Thessaloniki, Greece, were originally recruited for the present study. The subject sample was calculated according to the following: the subject (not the site) was chosen as the observational statistical unit, and a probing depth (PD) difference of 2 mm was chosen as a clinically desirable primary outcome. Therefore, in the current experiment, in order to detect such differences with a significance level (alpha) of 0.05 (two tailed), power of 80% (type beta) and an expected standard deviation of the after-before differences = 2, at least a sample size of 25 in each group is required (Statmate2®, Graphpad Inc, San Diego).

Subjects were diagnosed with generalized chronic periodontitis according to criteria described by the American Academy of Periodontology (Armitage, 1999). Further criteria for inclusion were: presence of at least 20 teeth, absence of antibiotic intake for the last three months, no known allergies to antibiotics and no periodontal treatment for the previous 12 months. Pregnant or lactating women were excluded from the present study. Smoking status (smoker, non smoker) as reported by patients was also recorded. The study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki, Greece and was approved by the Ethical Committee of the School of Dentistry.

The present study included one test and one control group characterized as: Group 1 - scaling and root planing plus Periochip® in four sites ≥ 5 mm and ≤ 7 mm; and Group 2 - scaling and root planing only, and was designed as a randomized, controlled clinical trial (RCT) according to the CONSORT criteria (Altman *et al.*, 2001).

The date of patient enrolment was recorded on a numbered list. Randomization was generated using random tables and the randomization list was kept by one of the authors (AK) until patients were eligible for the study. The study was designed as blinded concerning the examiner (MA) who was not aware of the treatment that the patient had received. Analysis of subgingival samples was performed by one of the authors (II) who was also unaware of the treatment that the patient had received (coded samples).

Upon final recruitment, subjects were scheduled for baseline sampling of subgingival plaque and for baseline full-mouth clinical recordings a week later, as described below.

All patients received detailed oral hygiene instructions and interdental toothbrushes and were provided with identical nylon, soft, multi-tufted toothbrushes and fluoride toothpastes. Toothbrushes were replaced every three months. Participants were instructed not to use any antiseptic mouthwashes throughout the study. Full-mouth scaling and root planing (SRP) was performed in two quadrants under local anaesthesia in two sequential visits, one day apart. Mechanical treatment included ultrasonic instrumentation (Piezon®, Instruments A and PS, EMS, Switzerland) followed by hand instruments (Gracey curettes SG 3/4, 11/12, 13/14, Hu-Friedy, Chicago, IL, U.S.A.). Duration of instrumentation for each visit ranged between five and 10 minutes per tooth. Scaling and root planing was performed by the same clinician (DS). Upon completion of treatment, in patients assigned to Group 1 (adjunctive Periochip®), a chlorhexidine chip was placed in four pre-selected pockets with probing depth and probing attachment level ≥ 5 mm and ≤ 7mm with a blunt instrument by the same clinician who performed SRP (DS). Care was taken to include interproximal sites of natural single-rooted teeth only, in all four quadrants, with adjacent teeth on both sides. Identical selection criteria were applied for four sites in patients assigned to Group 2, who did not receive any further treatment. Any adverse effects from chip placement were recorded.

Three weeks after completion of treatment participants were scheduled for subgingival sampling. Only subjects with proven ability to perform oral hygiene, as instructed (presence of plaque < 20% of surfaces) and weekly assessed, continued the study. Thus, three subjects were excluded at this time point and their data were not included in the analysis. Subsequently, three and six months after completion of treatment, patients were scheduled for microbial sampling and clinical recordings. Three participants (one from Group 1 and two from Group 2) were unwilling to complete the study. Subject data are presented in *Table 1* and the flowchart of patients throughout the study is illustrated in *Figure 1*.

Clinical recordings

Clinical data were recorded from all teeth present in the dentition. The following parameters were recorded at six sites for each tooth (disto-, mid- and mesiobuccal, mesio-, mid- and distolingual): a) probing depth (PD); b) probing attachment level (PAL); c) bleeding on probing (BOP).

Time points of recordings included baseline, three and six months after treatment. All measurements were performed by one calibrated examiner (MA) using a manual Williams probe (POW, Hu-Friedy, Chicago, IL) The examiner regularly performs clinical recordings in the Clinic of the Department and has reproducible assessments (Pearson's test, r = 0.971) as determined in 10% of her weekly registrations.

Table 1. Subject data

	Number	Age (mean ± SD)	Age range (yrs)	Male	Female	Smokers
Group 1	25	46.35 ± 7.31	36 - 64	14	11	5
Group 2	25	48.75 ± 10.15	37 - 75	13	12	4

Group 1 = adjunctive CHX chip; Group 2 = scaling and root planing only.

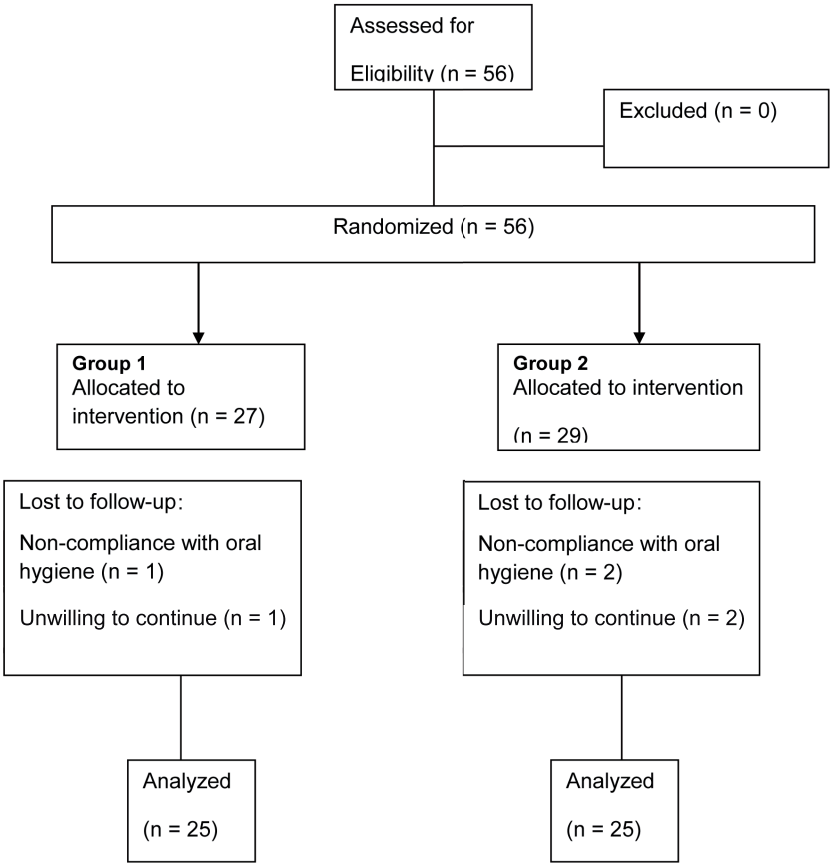

Figure 1. Flow-chart of patients through the experimental period.

Table 2. Clinical data (mean ± SD) of the two groups during the experimental period.

	Baseline		3 months		6 months	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Probing depth (mm)	5.62 ± 0.92 (a, b)	5.69 ± 0.67 (a, b)	3.79 ± 0.69 (a)	3.8 ± 1.02 (a)	3.83 ± 0.72 (b)	3.64 ± 0.79 (b)
Attachment level (mm)	6.47 ± 0.85 (a, b)	6.38 ± 1.0 (a, b)	5.46 ± 1.06 (a)	5.15 ± 1.47 (a)	5.07 ± 1.05 (b)	4.98 ± 1.37 (b)
Bleeding on probing	0.59 ± 0.35 (a, b)	0.66 ± 0.3 (a, b)	**0.48 ± 0.29**	**0.25 ± 0.24**	0.34 ± 0.32 (b)	0.33 ± 0.34 (b)

Numbers in bold display statistically significant differences between the two groups (ANOVA, $p < 0.05$). Numbers followed by the same letter differ significantly within groups (paired t-test, $p < 0.05$).

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Table 3. Bacterial counts x 10^5 (mean ± SE) during the experimental period

	Baseline		3 weeks		3 months		6 months	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Porphyromonas gingivalis	2.41 ± 0.56	2.58 ± 0.75(a, b)	0.32 ± 0.16	1.17 ± 0.47 (a)	1.11 ± 0.49	0.97 ± 0.47	1.32 ± 0.81	0.71 ± 0.26 (b)
Tannerella forsythia	2.8 ± 0.91 (a)	2.4 ± 1 (a)	3.07 ± 1.48	2.47 ± 1.33	3.58 ± 1.27	1.89 ± 0.87	1.06 ± 0.63 (a)	0.77 ± 0.33 (a)
Treponema denticola	2.72 ± 0.76 (a)	2.14 ± 0.57 (a)	2.94 ± 1.5	2.4 ± 1.37	1.2 ± 0.74	0.8 ± 0.5	1.38 ± 0.65 (a)	0.51 ± 0.25 (a)
Prevotella nigrescens	2.13 ± 1.05	2.01 ± 0.87	1.73 ± 0.75	2.78 ± 1.16	1.05 ± 1.41	1.41 ± 0.79	**0.66 ± 0.3**	**2.91 ± 1.06**
Prevotella intermedia	3.01 ± 0.97	2.73 ± 0.8	1.59 ± 0.92	3.57 ± 1.04	1.78 ± 0.7	1.54 ± 0.75	1.65 ± 0.74	1.26 ± 0.4=
Fusobacterium nucleatum	3.64 ± 0.65	3.83 ± 0.77	3.14 ± 1.25	3.91 ± 1.48	2.82 ± 0.9	3.14 ± 0.96	0.93 ± 0.34	3.41 ± 0.62
Campylobacter rectus	2.68 ± 0.59	1.37 ± 0.34	0.7 ± 0.49	1.81 ± 0.7	1.22 ± 0.55	1.94 ± 0.75	0.97 ± 0.38	1.73 ± 0.57
Veillonella parvula	3.15 ± 1.16	2.96 ± 0.92	1.51 ± 0.54	3.71 ± 1.5	2.52 ± 0.89	3.92 ± 0.81	**0.93 ± 0.44**	**2.38 ± 0.63**

Group 1 = adjunctive CHX chip; Group 2 = scaling and root planing only.
Numbers in bold display statistically significant differences between the two groups (Mann-Whitney test, $p < 0.05$).
Numbers followed by the same letter differ significantly within groups (Wilcoxon signed rank test, $p < 0.05$).

Microbiological examination

At all time points, microbial plaque samples were taken prior to the clinical measurements. Time points of sampling included baseline, three weeks, three and six months after treatment. Plaque samples were taken from pockets that received Periochip® (Group 1) and four pre-selected pockets with PD ≥ 5 mm and ≤ 7mm from each patient (Group 2). After isolating with cotton rolls, drying, and removal of supragingival plaque, subgingival samples were taken by means of a sterile Gracey curette, placed in 100 μl of TE buffer (Tris HCL 10 mM, EDTA 1 mM, pH 7.5) and stored after treatment in an alkali solution (0.5 M NaOH) at –20°C. A total of 800 samples was processed for eight bacterial species, using the "checkerboard" DNA-DNA hybridization technique as described in detail by Socransky *et al.* (Socransky *et al.*, 1994; 1998). The subgingival species used for development of digoxigenin-labelled whole genomic probes were *Porphyromonas gingivalis* (FDC 381), *Tannerella forsythia* (FDC 338), *Treponema denticola* (TD1), *Prevotella nigrescens* (VPI 8944), *Prevotella intermedia* (FDC 581), *Fusobacterium nucleatum ss vincentii* (FDC 364), *Campylobacter rectus* (FDC 371) and *Veillonella parvula* (ATCC 10790). Cell numbers were quantified by using software for array analysis (TotalLab TL100 v 2005, NonLinear Dynamics Ltd., Newcastle Upon Tyne, U.K.).

Statistical analysis

The statistical analysis of the data was carried out with the statistic package SPSS, 14.0 version. Probing depth was set as the primary outcome, and probing attachment level and bleeding on probing as secondary outcomes for the present study. Analysis was restricted only to participants who fulfilled the protocol in terms of eligibility, interventions and complete outcome assessments ("on-treatment" or "per protocol" analysis) as described in the CONSORT statement (Altman *et al.*, 2001).

Indicators of descriptive statistics were used, such as frequencies, percentage, average, variance and standard deviation for each group at all time points. To check differences between groups across all time points the general linear model, repeated measures procedure was applied with the patient as the observational unit. The ANOVA (analysis of variance) procedure was also implemented at each time point.

Levene's test for equality of error variances was applied in order to check for homogeneity of clinical parameters at baseline. Differences between time points within each group were separately tested with the paired samples *t*-test procedure. All the above were calculated for the subset of sites predetermined as test and controls and for the whole dentition.

Microbiological data were analyzed with the subject as the observational unit by applying non-parametric tests. Averaged bacterial numbers from each subject were consequently averaged for each group and compared at all time points. Homogeneity between the two groups at baseline was tested using the Kruskal-Wallis test. In order to identify specific differences between the two groups at each time point the Mann-Whitney test was applied. In order to identify differences between time points within groups the Wilcoxon signed rank test was applied. The significance level was set at 0.05 for all tests.

Results

No difference was observed between the mean ages of subjects in the two groups (*Table 1*, Mann-Whitney test, $p = 0.54$).

No adverse effects resulting from CHX placement, such as discomfort, pain or swelling, as reported by patients, were recorded.

The results of the present study concerning clinical data are presented in *Table 2*. All groups were homogeneous at baseline (Levene's test, $p > 0.05$) and no differences were observed between the two groups at any time point, with the exception of bleeding on probing at three months. Subjects treated with adjunctive CHX chips exhibited significantly higher bleeding scores as compared to control subjects (one-way ANOVA, $p < 0.05$). When comparisons were made within each group, both treatments resulted in significant improvement of all clinical parameters from baseline to three months, and this difference was maintained at six months from baseline in both groups (paired *t*-test, $p < 0.05$). No differences were observed between three and six months at both groups (paired *t*-test, $p > 0.05$). In addition, these parameters and comparisons were calculated for the full dentition with the subject as the observational unit and a similar pattern of differentiations was observed (data not shown).

Microbiological data for investigated species are presented in *Table 3*. High numbers were observed for both groups at baseline with no statistically significant differences between them (Kruskal-Wallis test, $p > 0.05$). Three weeks after completion of treatment a trend for reduction in numbers for both groups and most investigated species, but no differences between the two groups, were observed (Mann-Whitney test, $p > 0.05$). *P. gingivalis* was the only species that demonstrated statistically significant reduction from baseline for subjects who received SRP (Wilcoxon signed rank test, $p < 0.05$). Bacterial counts for investigated species were further numerically reduced for both groups at three months, but no differences were found between the two groups. At six months after completion of treatment, counts of *T. forsythia* and *T. denticola* were statistically significantly lower compared to baseline for both groups, while counts of *P. gingivalis* were statistically significantly reduced only for subjects who received SRP (Wilcoxon signed rank test, $p < 0.05$). No differences were observed between the two groups at the six-month time point other than for *P. nigrescens* and *V. parvula* (Mann-Whitney test, $p < 0.05$).

Discussion

Adjunctive effects of the CHX chip have been evaluated in a limited number of RCTs in the literature and, furthermore, few studies report on microbiological parameters. Currently, data in the literature (especially deriving from multi-centered studies) generally suggest beneficial effects of adjunctive Periochip® (Soskolne *et al.*, 1997; Jeffcoat *et al.*, 1998). However, these data have not been confirmed by other studies and, in addition, disparities in methodological issues such as study design, clinical criteria for selection, numbers of pockets included and times of application add to difficulties in interpreting results (Cosyn and Wyn, 2006). The present small, phase IV RCT was designed in order to investigate and compare the adjunctive effects of Periochip® at the initial treatment phase of chronic periodontitis on clinical and certain microbiological parameters. The main focus of this study was to provide information for clinical decision-making, and therefore the subject sample and desirable clinical outcomes were determined accordingly. Although large-scale studies (Soskolne *et al.*, 1997; Jeffcoat *et al.*, 1998) have shown additional pocket reduction when the chip was applied, which ranged between 0.30 and 0.46 mm, in everyday practice a number of clinicians favor the indiscriminate use of antimicrobials, and therefore information about a possible upper threshold of clinical gain is meaningful. It is well known that the proper choice of the sample size is always a very sensitive, though crucial, issue. The present study was designed with the subject as the observational unit and a targeted PD difference of 2 mm. The chosen PD difference would overcome any errors in measurements and also allow for "safer" extrapolation of clinical significance (Persson, 2005). Although an important effect of SRP on indices of periodontal inflammation is anticipated, findings from the present study provide additional information to clinicians about the clinical and microbiological impact of adjunctive CHX chip at the initial treatment phase. According to the design of the present study, only subjects who complied well with proper oral hygiene procedures (Xajigeorgiou *et al.*, 2006), as instructed in detail, were included in the two groups, and three subjects were excluded for non-compliance. In addition, as mentioned in the materials and methods section, three participants were unwilling to continue the study. This approach, although arguably a caveat of the present study design, was chosen as reflecting a "real-life" situation due to various possibilities, including the motivation of participants to perform better hygiene immediately before visits and the transient nature of supragingival plaque accumulation, which may lead to erroneous recordings at single-point visits, especially when applying a dichotomous scoring (Hancock and Newell, 2001). The issues of correctly measuring supragingival plaque in clinical trials, the inability of current plaque indices to assess subgingival accumulation, and even the effect of personal hygiene on chronic periodontitis remain unresolved (Goodson, 1986; Lindhe, 1986; Hujoel *et al.*, 2005; McCracken *et al.*, 2006). Therefore, according to the design of the present study, by including microbiological assessments we obtained information about the subgingival plaque of the sites under investigation and the impact of therapy on consensus periodontal pathogens. It is worth men-

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tioning that the endpoint of this clinical study was set at six months from completion of treatment because it was considered that extending the experimental period without further mechanical treatment might present risks of recurrence of the disease. In addition, we were unable to stratify our subjects according to smoking status because of the small number of smokers.

In agreement with other studies, the major effect on clinical parameters for both groups resulted at three months after treatment and this effect was maintained at six months (Badersten *et al.*, 1981; Haffajee *et al.*, 1997). We did not include clinical recordings at three weeks because even if early effects of the CHX chip were missed, longer-term impacts are required for clinical significance. When comparing differences between the two groups at different time points, no differences were observed concerning clinical parameters other than lower bleeding on probing scores at three months for the control group (*Table 2*), and the targeted PD difference of 2 mm between the two groups set as primary outcome of the present trial was not achieved. According to the study design, SRP was performed by the same clinician, and only single-rooted teeth and interproximal areas with comparable periodontal destruction (PD and PAL) were included in both groups in order to reduce possible discrepancies concerning subgingival instrumentation. Therefore, according to the findings and under the limitations of the present study, it is suggested that at least at the initial mechanical treatment phase, clinicians should not expect to achieve an adjunctive effect of the chip reaching a PD difference of 2 mm compared to thorough SRP alone.

In contrast to these findings, studies evaluating adjunctive Periochip® in residual pockets after the initial treatment phase have shown statistically significant clinical improvements, suggesting that this phase of periodontal treatment might be more appropriate for applying the CHX chip (Heasman *et al.*, 2001; Salvi *et al.*, 2002; Soskolne *et al.*, 2003).

Microbiological data with respect to the CHX chip are very confined and should be interpreted with caution due to small samples, varying length of observation and methodological differences (Daneshmand *et al.*, 2002; Grisi *et al.*, 2002; Mizrak *et al.*, 2006). In the present study, we monitored 200 sites throughout the experimental period and we included a sampling point at three weeks in order to investigate short-term effects of adjunctive Periochip® compared to SRP. High prevalence and levels of investigated species were observed in subgingival samples at baseline for both groups (*Table 3*). Both treatments resulted in reduction of bacterial counts, which reached statistical significance at six months for the important pathogens *T. forsythia* and *T. denticola* (*Table 3*). Only control subjects displayed statistically significant reduction of *P. gingivalis* at 3 weeks and 6 months after treatment. Our findings are indicative of the difficulty of mechanical treatment to predictably eliminate or suppress the microbial factor. As already shown in the literature (Shiloah and Patters, 1994; Haffajee *et al.*, 1997) and pointed out by relevant reviews (Petersilka *et al.*, 2002; Umeda *et al.*, 2004; Teles *et al.*, 2006), major periodontal pathogens, notably members of the "red complex," appear to be affected by mechanical treatment for up to 12 months. Some reports demonstrate few benefits of SRP for certain species, at least on a subject basis (Beikler *et al.*, 2004; Darby *et al.*, 2005; Doungudomdacha *et al.*, 2001). The modification of the pocket environment over time induced by subgingival instrumentation is suggested to be an important factor for sustaining lower numbers of pathogens, and this fact may account for the statistically significant reductions of important pathogens observed in our study not earlier than six months. Within the confines of this small phase IV RCT, we failed to detect any inter-group differences in "red-complex" bacteria at three weeks, three months or six months post-treatment. We did detect significantly lower levels for one "orange-complex" bacterium (*P. nigrescens)* and one "lavender-complex" bacterium (*V. parvula)* for subjects treated with adjunctive CHX chip as compared to subjects treated with scaling and root planing alone. These data, in general, are consistent with previous studies that demonstrated no short-term (2 to 4 weeks) or long-term (3 to 9 months) differences for the major "red-complex" pathogens with adjunctive CHX chip by employing culture or a commercially available test (Daneshmand *et al.*, 2002; Grisi *et al.*, 2002). Several explanations for these unexpectedly minor effects of the CHX chip have been reported in the literature. These include poor adherence of CHX on the root surfaces, interference of the device itself with the healing process, or inactivation of the active ingredient from bacterially derived substances (Cosyn and Wyn, 2006).

It is therefore suggested that the concentrations of CHX achieved in the subgingival environment (Soskolne *et al.*, 1998) after placement of the chip might not be sufficient for the satisfactory inhibition of periodontal pathogens as previously reported (Stanley *et al.*, 1989), especially given the nature of their biofilm structure.

Conclusions

In this small, six-month, phase 4 trial, no differences in mean probing depth reduction or "red-complex" periodontal pathogens were detected for patients with chronic periodontitis treated with adjunctive chlorhexidine chip (single administration) as compared to patients treated with scaling and root planing alone.

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