Sustained Local Delivery of Chlorhexidine During Initial Therapy of Chronic Periodontitis

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Controlled local delivery of disinfecting agents has been demonstrated to be efficient in improving the outcome of periodontal therapy. The use of PerioChip® as an adjunctive to scaling and root planing (SRP) has been reported to improve the result of local therapy. In this study, the effect of the chip on clinical and microbiological parameters prior to SRP was analyzed.

The following clinical parameters - probing pocket depth (PPD), attachment level (AL) and bleeding on probing (BOP) - were recorded at six sites/tooth in 14 patients with chronic periodontitis. Subgingival samples were collected from the deepest periodontal pocket (total: 56 sites) and analyzed by means of polymerase chain reaction (PCR). Chlorhexidine chips were then inserted into these pockets and the clinical and microbiological recordings were repeated on the 10th and 28th day post-placement.

At baseline, *Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), and Treponema denticola (T.d.)* were the most frequently detected pathogens (95%, 98% and 81% of sites respectively). The use of PerioChip® resulted in significant ($p<0.05$) clinical improvements (mean PPD reduction: 0.96 mm; reduction in the percentage of bleeding sites: 53%) and in a significant reduction in the frequency of P.g., T.f. and T.d. (95 to 15%, 98 to 35% and 81 to 11%) respectively.

It is concluded that the local disinfection of periodontal pockets with PerioChip® prior to mechanical therapy is effective in reducing putative pathogens in the pockets and also in improving clinical signs of periodontitis. However, sole use of PerioChip® without subsequent SRP cannot be recommended.

INTRODUCTION

Periodontitis is an infectious disease characterized by inflammatory changes in the surrounding tissues leading to periodontal attachment loss and alveolar bone destruction. As dental caries has declined, periodontitis has become the most common reason for tooth loss in adults (Listgarten, 1986; Page et al, 1997).

The current diagnostic model suggests that the development of disease requires both a susceptible host and a pathogenic flora (Wolff et al, 1994). Host susceptibility appears to be related to genet-ic and behavioral factors such as smoking (McDevitt et al, 2000). Pathogenic bacteria include predominantly gram-negative anaerobes, among which certain pathogens are strongly associated with the disease (Haffajee et al, 1988; Haffajee and Socransky, 1994; Slots, 1977; Socransky and Haffajee, 1997). Since 1979, the putative periodontopathic bacteria *Actinobacillus actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Prevotella intermedia (P.i.) and Treponema denticola (T.d.)* have been reported to be strongly associated with disease and disease activity (Slots, 1979).

Each of these species displays potential virulence factors (e.g. leukotoxins, high levels of protease activity, tissue invasion), which could contribute to the loss of attachment that occurs as periodontitis progresses (Brown et al, 1994; Fives-Taylor et al, 1999; Lotufo et al, 1994; Mintz and Fives-Taylor, 1999; Paju et al, 2000; Van Winkelhoff et al, 1986; Vayssier et al, 1994).

A.a. has been shown to possess a myriad of virulence factors that enable it to circumvent the host's protective strategies (Fives-Taylor et al., 1999). Many of these virulence factors may be involved in the pathogenesis of the disease.

Also, P.g. has long been considered an important member of the periodontopathic microbiota involved in periodontal disease progression and bone and tissue destruction (Rodenburg et al, 1990; Van Winkelhoff et al, 1988; Van Winkelhoff et al, 1993; Van Winkelhoff et al, 2002).

P.i. is frequently associated with periodontal disease, specifically chronic periodontitis and acute necrotizing ulcerative gingivitis (Fukui et al, 1999), and has been frequently isolated from deep periodontal pockets (Dahlen et al, 1990b; Dahlen et al, 1990a; Riggio et al, 1998).

A.a., P.g., and P.i. occur in significantly greater proportions in subjects and sites with recent periodontal disease activity (Haffajee and Socransky, 1994; Rams et al, 1996; Slots and Listgarten, 1988).

Furthermore, it has been reported that subgingival proportions of P.g. and P.i. which are above 2% of the recoverable flora are significant predictors of progressive periodontitis over an 18-month period in older adults (Brown et al, 1994).

In addition, T.f. has been thought to play an etiological role in chronic periodontitis since 1979 (Tanner et al, 1998; Tanner et al, 1986). This organism has also been associated with the advanced and refractory forms of the disease (Dzink et al, 1988; Haffajee et al, 1991; Lai et al, 1987; Listgarten et al, 1993; Zambon et al, 1996). T.f. produces a trypsin-like enzyme and a sialidase that may be of importance in pathogenesis (Lotufo et al, 1994). Moreover, Wennstrom et al (1987) showed that the absence of A.a., T.f. and P.i. can be considered as a significant predictor of no further periodontal disease activity.

Microbiological studies have also shown that T.d. quantitatively relates to the severity of periodontal inflammation by harboring a variety of potential virulence properties (Arakawa and Kuramitsu, 1994; Ashley et al, 1988; Baehni et al, 1992). These include suppression of lymphocyte blastogenesis as well as the inhibition of fibroblast and polymorphonuclear leukocyte function (Wyss et al, 1999).

The elimination of these periodontopathogens was found to have a major impact on treatment outcome (Slots and Rams, 1990), and therefore they have become the target organisms during periodontal therapy.

Several methods have been described for the reduction/eradication of these bacteria: scaling and root planing (SRP), surgical procedures and the use of antibiotics, both systemically as well as by means of local delivery systems (Erverdi et al, 2001; Flemmig et al, 1998; Rothstein, 1999; Vandekerckhove et al, 1997; Vandekerckhove et al, 1998).

In addition, many investigators have used various disinfecting agents either as a rinse or in oral irrigation devices as a pre-operative measure (Brenman and Randall, 1974; Cutcher et al, 1971; Huffman et al, 1974; Randall and Brenman, 1974; Scopp and Orvieto, 1971; Soskolne, 1997; Winslow and Millstone, 1965; Witzenberger et al, 1982). Among these agents, chlorhexidine (CHX) is known to be an effective antimicrobial and has been used as a topical antiseptic for over 30 years (Hugo, 1967; Russell, 1969). It is an agent with cationic characteristics that can attach to the negatively charged bacterial cell membrane and disrupt it. Moreover, CHX prevents bacteria from attaching to each other, or to a surface (Hennessey, 1977; Hennessey, 1973). Several studies and reviews reported that CHX is an effective antimicrobial mouth rinse (Addy, 1986; Bonesvoll and Gjermo, 1978; Loe and Schiott, 1970; Mackenzie et al, 1976).

In order to reach the subgingival environment, pocket-irrigation with a syringe is indicated (Gjermo et al, 1974; Soh et al, 1982).

Since local delivery of antimicrobial agents could eliminate the infection without the need of subjecting the entire body to unnecessary exposure to a drug, which can lead to bacterial resistance (Roberts et al, 2000), both non-degradable and degradable local delivery systems have been developed (Kanamoto et al, 2000; Wynn et al, 1999).

However, a large body of evidence has demonstrated failure of subgingival irrigation with CXD to

impact on periodontal outcomes (Chapple et al, 1992; Quiryenen et al, 2000). Sixteen years ago, Stabholz et al (1986) used a controlled-release device to deliver CHX in 13 pockets of eight patients. They reported that CHX was effective against subgingival plaque bacteria and that the microbial effects were evident for up to 11 weeks after treatment (Stabholz et al, 1986).

Recently, more studies have confirmed the effects of a controlled-release local-delivery device of CHX (Jeffcoat et al, 1998; Killoy, 1998b; Soskolne et al, 1997; Soskolne et al, 1998).

A biodegradable chip for controlled and sustained and direct delivery of CHX to the periodontal pocket has been developed (Steinberg et al, 1990) and studied in several multicenter randomized controlled clinical trials (Anonymous, 1997; Roberts et al, 1998). Soskolne et al (1998) reported that the use of CHX-Chip (PerioChip®) in conjunction with SRP, when compared to SRP alone, significantly improved the clinical parameters of probing attachment level and bleeding on probing.

They also concluded that the use of the CHX-Chip can be most effective when it is placed every three months in pockets that remain > 5 mm deep after completion of periodontal therapy.

In an in vitro study, Stanley et al (1989) reported that a CHX concentration of 125 µg/ml inhibited the growth of 99% of the pocket microflora. Interestingly, in their pharmacokinetic study, Soskolne et al (1998) analyzed the release of CHX of the CHX-Chip (PerioChip®) and reported an average of CHX concentration in crevicular fluid greater than 125 µg/ml for eight days. In addition, it was indicated that the chip could maintain clinically effective levels of CHX within the gingival crevicular fluid (GCF) of periodontal pockets for over one week with no detectable systemic absorption and no chip residue in any of the pockets (Soskolne et al, 1998).

However, with the exception of the above mentioned investigation of Stabholz et al (1986), very little data is available about the microbiological effect of the CHX-Chip on the subgingival flora (Stabholz et al, 1986).

The aim of this study was to evaluate the effect of the (PerioChip®) on the subgingival microbiota, i.e. the periodontopathogens: A.a, P.g., P.i., T.f. and T.d, prior to SRP, as well as to monitor this effect on clinical parameters (PPD and BOP).

STUDY DESIGN AND RESULTS

Subjects

Fourteen volunteers with chronic periodontitis (mean age 45 years ± 7.8) were included in this randomized, single-blinded study. They consulted the Department of Periodontology as a consequence of severe chronic periodontitis.

All patients fulfilled the following inclusion criteria: all of them were older than 35 years, non-smokers, without systemic diseases, did not use antimicrobials during or within the three months preceding the study, had at least three teeth with PPD > 5 mm which bled when probed. All demonstrated reasonable oral hygiene.

Clinical Examination

The participants were instructed about the study and an informed consent form was obtained. Each patient was given a code number, which was used to identify him throughout the study. Investigators and lab staff were blinded to that coding.

At baseline (day 0), the patients were subjected to an initial clinical examination which included the following parameters: PPD, AL, BOP at six sites/tooth using a PCP UNC 15 probe. Oral hygiene was assessed by the papillary bleeding index (PBI) and a plaque index (PLI) (Saxer and Mühlemann, 1975).

Subsequently, the patients were instructed on an individual basis how to perform proper oral hygiene. Following this their teeth were professionally cleaned supragingivally.

The CHX-chips were then inserted into the deepest four pockets of each patient (total: 56 sites) by means of cotton forceps, and all of the above mentioned clinical parameters as well as oral hygiene (PLI and PBI) were repeated on days 10 and 28 post chip placement.

Microbiological Parameters

Immediately prior to chip placement, subgingival plaque was sampled using sterile paper points (ISO size 45, Antaeus, Vereinigte Dentalwerke, Munich, Germany) from the same sites planned to receive the CHX-chip. Prior to sampling, the selected sites were cleaned supragingivally using sterile curettes, isolated from saliva with cotton rolls

Table 1 Species-specific primers according to (Ashimoto et al, 1996)
A. a. = Actinobacillus actinomycetemcomitans, P. g. = Porphyromonas gingivalis, P. i. = Prevotella intermedia,
T. f. = Tannerella forsythia, T. d. = Treponema denticola

Primer	Sequence	Position and Length of Sequence
A.a.-F A.a.-R	AAA CCC ATC TCT GAG TTC TTC TTC ATG CCA ACT TGA CGT TAA AT	478–1034 (557)
P.g.-F P.g.-R	AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	729–1132 (404)
P.i.-F P.i.-R	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	458–1032 (575)
T.f.-F T.f.-R	GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACCT	120–760 (641)
T.d.-F T.d.-R	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	193–508 (316)

and gently dried in order to prevent contamination (Berger et al, 1994). The paper points were kept in place for 20 s (Hartroth et al, 1999) and then transferred into an empty pre-labeled screw-capped vial. All samples were coded in such a way that neither the sites nor the time of sampling could be detected, ensuring that the microbiological analysis was blinded. Subgingival sampling was then repeated on days 10 and 28 post chip placement.

The microbiological analysis was performed in the Institute of Microbiology at our university by means of PCR for each of the following pathogens: A.a., P.g., T.f., P.i. and T.d..

Microbiological Analysis

Sample Transport

Samples were kept dry at room temperature and submitted to the Institute of Microbiology at different time intervals. Since samples were not obtained for culturing, no transport medium was required and storage for several weeks was possible without affecting the sensitivity of the molecular analysis. This was previously tested before the start of the study (Hossain et al, 2005).

DNA Extraction

The DNA was extracted using a newly developed method for the extraction from paper points. In brief, after addition of NaOH, aqua-dest and four glass beads, samples were thoroughly vortexed, warmed at 95°C for 15 minutes and centrifuged. Five μL of the supernatant was then transported to a vial containing 95 μL of reaction mixture for PCR analysis.

Each of the reaction mixtures contained 83.3 μL aqua dest, 10 μL of PCR-buffer, 0.5 μL Taq-DNA polymerase (Life Technologies, Karlsruhe, Germany) 0.2 μL of each of the dNTPs and 0.2 μL of each the forward and reverse primer specific for one pathogen.

PCR-Primers

Specific primers for A.a, P.g., P.i., B.f. and T.d. described by Ashimoto (1996) were used for the PCR detection (Table 1) (Ashimoto et al, 1996). The sensitivity of the PCR detection was 20 to 80 cells (Hossain et al, 2005).

The determination of the bacterial load was performed on a semiquantitative base in classifying the thickness of the bands in the agarose gel, assigning 1 = low, 2 = medium and 3 = high number of bacteria in the sample.

Fig 1 Mean pocket probing depth (mm) of all sites on day 0, 10 and 28. Chips were inserted on day 0. Significant decrease with (p<0.05) White rhombi = median values with standard deviation (n=48).
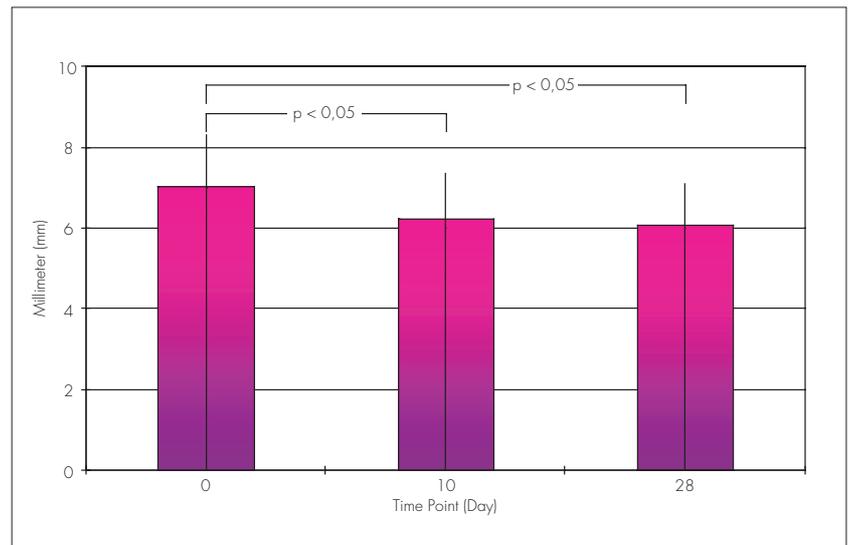
Statistical Evaluation

Four teeth per patient were included in the analysis. All data were stored in a personal computer using Excel software (Microsoft, Unterschleissheim, Germany) and checked in order to exclude false and identify missing values.

The sum of all probing depths per tooth and the sum of bleeding upon probing per tooth was calculated for each time point.

For each bacterial species the median of the scores of all four teeth was calculated. The following hypotheses were tested: H_0 = there are random changes in the number of bacteria in the subgingival samples which are independent from any time point; H_1= any changes observed are dependent upon the time and treatment. Since the analysis was based on scores, the Friedman rank sum analysis of variance was used for the statistical testing. Afterwards, based on the rank sums distribution-free comparisons at the different time points were calculated and corrected for multiple comparisons.

Statistical analysis was performed in the Institute of Medical Informatics using SAS version 8.02 (Cary, North Carolina, USA).

RESULTS

Clinical Data

At baseline, 95% of the sites that received the chip bled on probing and had a mean PPD of 7.043 ± 1.253 mm (Figs 1 and 2).

Chip placement were easy and was achieved in a relatively short time of one to two minutes. A total of 56 chips was used and only one had to be removed after placement. No chip residues were detected clinically 10 days after application. Furthermore, none of the patients reported any objective or subjective adverse events regarding chip application. Ten and 28 days post chip placement, a clinical improvement was observed in sites that had received the CHX chip (mean PPD reduction: 0.7968 mm ± 0.9 mm and 0.8296 mm ± 0.88 mm respectively). Furthermore, the results showed a significant decrease in the percentage of bleeding sites throughout the study (60%, and 52% on days 10 and 28) (Figs 1 and 2, Table 3a and 3b). Interestingly, the results showed that a significant clinical improvement including PPD and BOP occurred in treated sites demonstrating an initial PPD > 8 mm (mean PPD reduction: 1.8 mm ± 1.2 mm (day 10), 2 mm ± 0,6 mm (day 28). BOP decreased at these sites from 100% (day 0) to 16.7% (day 10) to 33.3% (day 28) (Table 2).
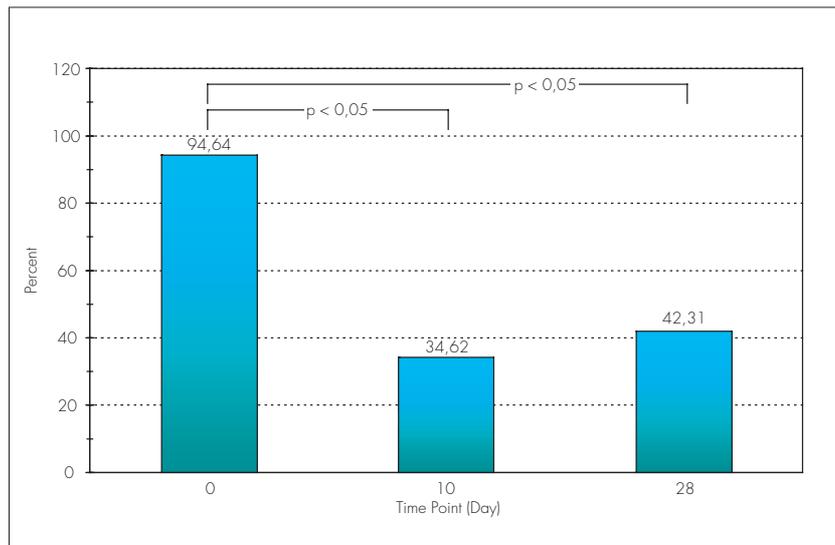
Fig 2 Percentage of bleeding sites (after probing) on day 0, 10 and 28. Significant decrease (p<0.05) (n=56).

Table 2 Mean probing depth and percentage of bleeding sites of deep pockets (initial PD>8 mm) before (day 0) and after chip placement (n=6).

Parameter	Day 0	Day 10	Day 28
ST: MW ± SD	9.4 ± 0,52	7.62 ± 0,84	7.38 ± 1.0
% bleeding sites	100	16.7	33.3

Tables 3a and b Difference in a) pocket probing depth (PPD) b) bleeding upon probing (BOP) on the different time points.

Table 3a Mean PPD, Comparison of day 0, 10, 28. The critical rank sum difference (Crit. Difference) is based on an error probability of p=0.05.

Time Point 1	Time Point 2	RS-Difference	Critical Difference	Significance
0	10	10	9.37	p < 0.05
0	28	12.5	9.37	p < 0.05
10	28	2.5	9.37	–

Table 3b Mean BOP, Comparison of day 0, 10, 28. The critical rank sum difference (Crit. Difference) is based on an error probability of p=0.05.

Time Point 1	Time Point 2	RS-Difference	Critical Difference	Significance
0	10	13.5	10.48	p < 0.05
0	28	13.5	10.48	p < 0.05
10	28	0	10.48	–

Table 4 Differences between different time points for all scores of the different bacterial species
Comparison of day 0, 10, 28. The critical rank sum difference (Crit. Difference) is based on an error probabbility of p=0.05, RS-Difference= Difference of rank sums.

Species	Time Point 1	Time Point 2	RS-Difference	Critical Difference	Significance
P. g.	0	10	96	38.39	p < 0.05
P. g.	0	28	122	38.39	p < 0.05
P. g.	10	28	26	38.39	–
P. i.	0	10	82.5	38.39	p < 0.05
P. i.	0	28	89	38.39	p < 0.05
P. i.	10	28	6.5	38.39	–
A. a.	0	10	1	38.1	–
A. a.	0	28	2	38.1	–
A. a.	10	28	1	38.1	–
T. d.	0	10	109	38.39	p < 0.05
T. d.	0	28	111	38.39	p < 0.05
T. d.	10	28	2	38.39	–
T. f.	0	10	109.5	38.39	p < 0.05
T. f.	0	28	127	38.39	p < 0.05
T. f.	10	28	17.5	38.39	–

Microbiological Data

On day 0, molecular analysis showed that 98% of the sampled sites harbored T.f., 97% P.g., 89% P.i., 6% A.a. and 87% T.d. (Fig 4). Moreover, 94% of the pockets were contaminated with at least three of these putative pathogens. Presuming that a microbiological improvement can be defined as a total eradication or alternatively a reduction in the quantity of bacteria, our results clearly indicated that 28 days post chip-placement 95% of the sites presented significant microbial improvement (Table 4).

The application of the chip resulted in a significant reduction of the percentage of sites harboring P.g., T.f., P.i. and T.d. (Fig 3).

Moreover, a semi-quantification of the PCR products, using band density from the PCR gels, implies that the number of pathogens in colonized sites decreased when compared to pre-chip placement (Fig. 4a–e).

DISCUSSION

A total of three different multicenter studies with the CHX chip were conducted in Europe at three (Soskolne et al, 1997) and in USA at 10 centres (Jeffcoat et al, 1998).

In the first randomized, single blind, split mouth case-control design of six months duration the SRP plus chip group showed significantly greater reduction in PPD and improvement in AL compared to the SRP group alone. Moreover, a significant difference in the BOP scores favoring SRP plus chip at the three-month interval was reported.

The other two randomized, double blind, trials of nine months duration reported similar results, for PPD, AL and BOP for the chip group compared to the placebo one. Furthermore, no patient treated with SRP plus CHX chip lost bone over the nine months, while 25% of the patients from the SRP group showed alveolar bone loss in one or more sites over the same time period.

These results, reviewed and discussed by Killoy (1998a), were explained by the suppression of the subgingival flora induced by the CHX released
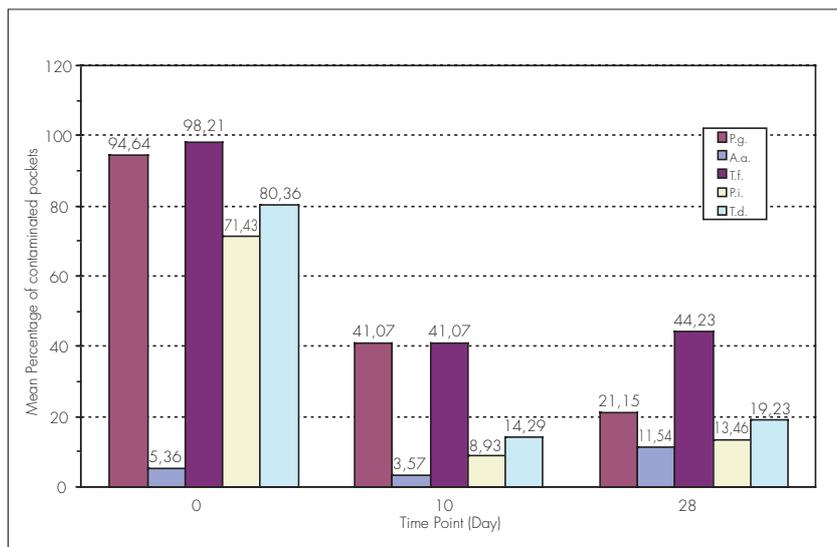
Fig 3 Mean percentage of different species in the pockets before (day 0) and after (day 10, 28) chip placement.
A.a.= Actinobacillus actinomycetemcomitans
P.g.= Porphyromonas gingivalis
P.i.= Prevotella intermedia
T.d.= Treponema denticola
T.f.= Tannerella forsythia.
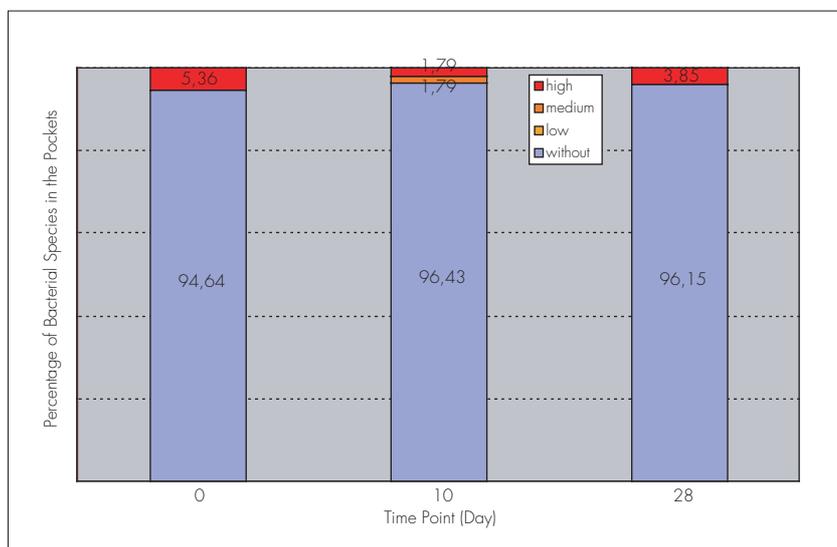
Figs 4a to e Relative amounts of different species (4a-4e) in the pockets before (day 0) and after (days 10, 28) chip placement . Scoring was performed according to thickness of the bands in the agarose gels (for details see text).

Fig 4a Relative amount of *A.a.*
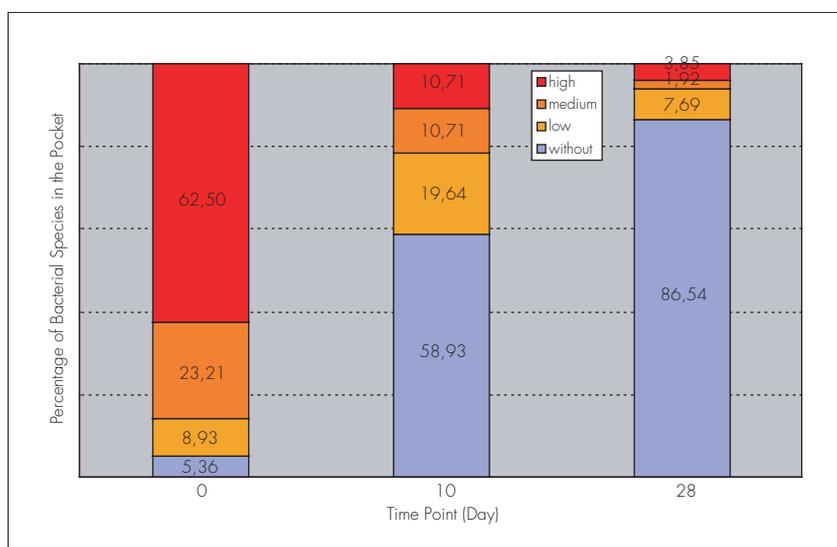
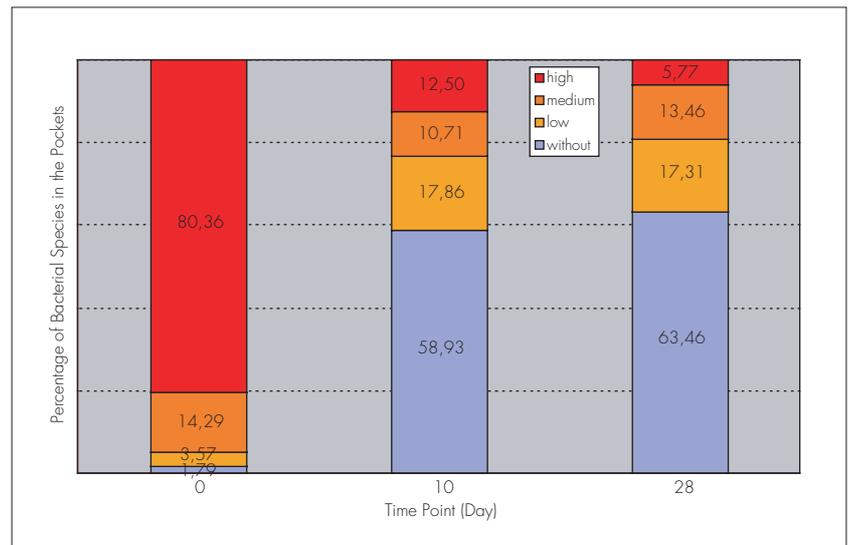
Fig 4b Relative amount of *P.g.*
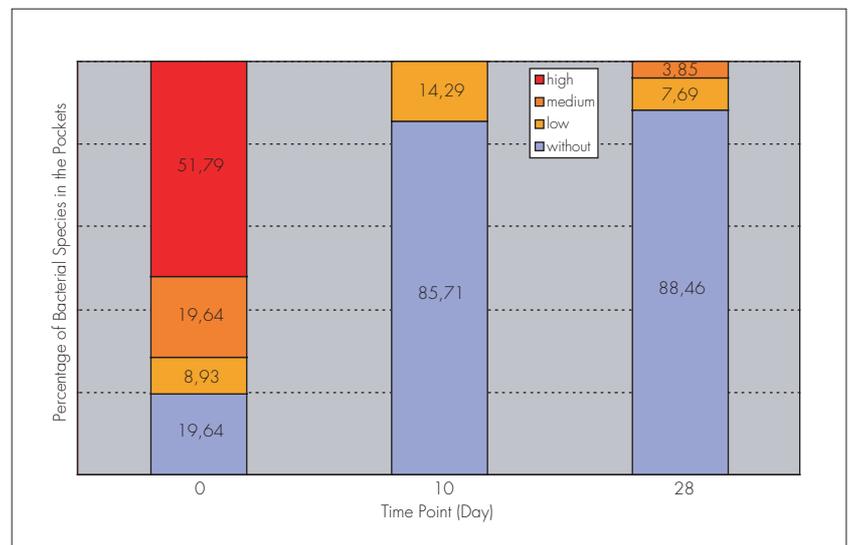
Fig 4c Relative amount of *T.f.*

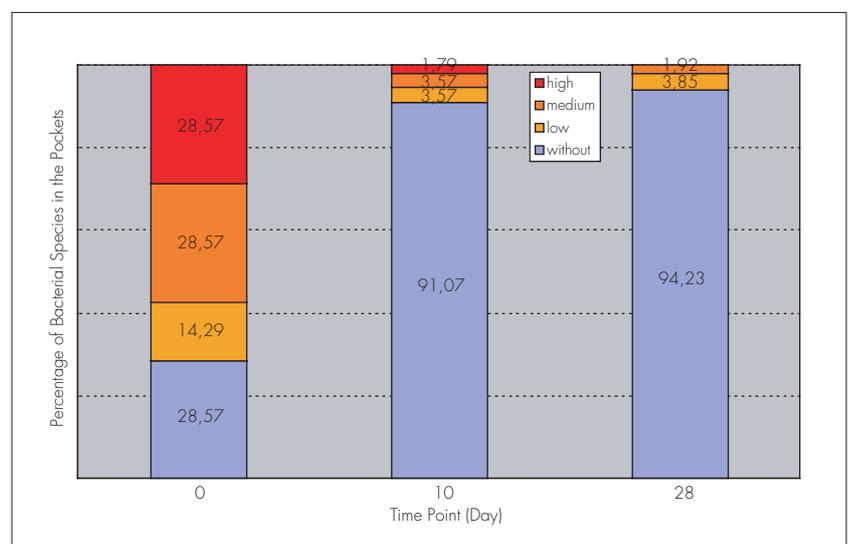**Fig 4d** Relative amount of *T.d.*

Fig 4e Relative amount of *P.i.*

from the chip. However, a microbiological analysis to monitor these changes has not been performed in these studies (Killoy, 1998b).

In the present study, the influence of the PerioChip® on the subgingival microbiota of 14 patients harboring at least two of five major putative periodontopathogens - A.a., P.g., T.f., P.i. and T.d. – has been demonstrated. In addition, differences in clinical parameters (PPD and BOP) were reported. Our results indicate that the CHX released from the chip significantly reduced the levels of all of these pathogens in some of the treated sites below detectable levels (20-100 cells).

PerioChip® treatment resulted not only in a significant reduction in the number of colonized sites, but it also appeared to reduce the concentration of pathogens remaining in some colonized sites.

The success of local periodontal treatment is not only reflected in the improvement of the clinical parameters, but is also measured by changes in the microbiological composition of the subgingival flora. A substantial reduction of the commensal flora or a complete eradication of the periodontopathogens in the subgingival flora should be obtained (Bollen and Quirynen, 1996). In recent years, Socransky et al. (Socransky and Haffajee, 1992) have modified the classical Koch's postulates (Koch, 1882) required a given specific bacterium to be considered as a periodontal pathogen. One important characteristic is that the pathogen should be present in high concentrations at diseased sites and absent in non-diseased sites. Our data indicate that the number of remaining pathogens in treated sites was significantly lower than it was before chip application. Thus, it can be concluded that an obvious substantial decrease in the number of pathogens in these sites took place.

The elimination or the reduction of these organisms was found to have a major impact on the treatment outcome (Bollen et al, 1996; Slots et al, 1990). This is of specific importance if we consider other systemic mechanisms initiated by oral pathogens.

Bacteremia caused by SRP, subgingival irrigation or other dental procedures such as extraction, endodontics or surgery (Cutcher et al, 1971; Mulligan, 1980; Winslow and Millstone, 1965) has been well documented. Furthermore, periodontal pathogens have been detected in atherosclerotic plaques (Deshpande et al, 1998;

Zambon, 1996), and accumulating evidence suggests that periodontitis and periodontal pathogens are risk factors for cardiovascular disease, stroke and low birth weight (Deshpande et al, 1998; Dorn et al, 1999; Lofthus et al, 1991; Offenbacher et al, 1999; Waki et al, 1990).

One of the limitations of our study is the lack of matched-control sites within the study subjects. It is possible that the supragingival plaque removal by sterile currette prior to paper point sampling of the subgingival microbiota, along with the improved oral hygiene, may have been responsible for some of the improvements in clinical and microbiological outcomes. However, this is unlikely to have been a major factor in this study.

These short-term results suggest that the application of the chip in acute inflamed pockets prior to local mechanical treatment can help to reduce the bacterial load and may also reduce the risk of transient bacteremia.

CONCLUSIONS

Killoy (1998) suggested three different phases in periodontal treatment where a local delivery system could be used to help control the patient's periodontitis: in the anti-infective phase as an adjunct to SRP, at re-evaluation following SRP and during maintenance. Due to its efficacy and ease of use, the chlorhexidine chip was suggested to be the method of choice in any of these phases (Killoy, 1998a; Killoy and Polson, 1998).

In this investigation, we introduced another approach to demonstrate the disinfective efficacy for the CHX-chip (PerioChip®) in order to protect the host. Further studies are required to substantiate these findings.

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