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Debridement and local application of tetracycline-loaded fibres in the management of persistent periodontitis: results after 12 months

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Abstract

Backgrounds, aims: The aim of our study was to evaluate the clinical, radiological and microbiological response to the local delivery of tetracycline (TE) of sites with persistent periodontal lesions.

Material and Methods: The study was conducted in a split-mouth design. Nineteen patients with at least four bilateral pockets 4–5 mm and bleeding on probing (BOP) were treated with scaling and root planing (SRP) plus TE fibres (test sites) or with SRP alone (control sites). Clinical and radiological measurements were taken at baseline, 6 months and 12 months post-operatively. Subgingival plaque samples were collected at baseline, at fibres removal, 6 and 12 months following treatment and analysed by polymerase chain reaction.

Results: Both treatments yielded a statistically significant ($p < 0.05$) reduction of probing depth (2.05 and 1.21 mm), gain of clinical attachment level (1.71 and 0.53 mm) and reduction of BOP scores (23.68% and 57.89%) for TE and SRP groups, respectively, when comparing 12-month data with baseline. The differences between two groups were significant. The prevalence of *Treponema denticola* and *Bacteroides forsythus* decreased after therapy in both groups but only in the test sites *Actinobacillus actinomycetemcomitans* and *Prevotella intermedia* were not yield detected. The pathogens could be eliminated from five periodontal pockets by SRP alone, while 21 TE sites were not recolonized at 12 months.

Conclusions: SRP plus TE fibres gave the greatest advantage in the treatment of periodontal persistent lesions at least 12 months following treatment.

Key words: *Actinobacillus actinomycetemcomitans*; *Bacteroides forsythus*; periodontal disease/microbiology; periodontal disease/therapy; *Porphyromonas gingivalis*; *Prevotella intermedia*; scaling and root planing; tetracycline fibre; *Treponema denticola*

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Owing to the fact that periodontitis is a multifactorial disease in which bacteria play an essential role, the control of its prevalence and progression requires a reduction of subgingival microbial plaque mass or at least a suppression of periodontopathic bacteria (Vandekerckove et al. 1997). Conventional scaling and root planing (SRP) in conjunction with plaque control results in an alteration of the subgingival environment that is sufficient, in most instances, to improve periodontal health and to arrest further loss of attachment (Cobb 2002). Nevertheless, SRP may not predictably lead to a complete elimination of the disease (Kaldahl et al. 1993, Haffajee et al. 1997a): in some patients who are otherwise responsive to plaque-focused therapy, a fraction of the affected sites do not stabilize and gingival inflammation persists or recurs (Kornman 1996). When periodontitis progresses despite SRP, high levels of putative pathogens, such as *Actinobacillus actinomycetemcomitans* (Van Winkelhoff et al. 1992, Mombelli et al. 2000), *Porphyromonas gingivalis* and *Prevotella intermedia* (Sato et al. 1993, Haffajee et al. 1997b, Chaves et al. 2000, Mombelli et al.

2000), *Bacteroides forsythus* (Taubman et al. 1992, Listgarten et al. 1993, Haffajee et al. 1997b), *Fusobacteruim nucleatum* (Sato et al. 1993) and *Treponema denticola* (Simonson et al. 1992, Haffajee et al. 1997b) are found in subgingival plaque. In the management of localized non-responsive sites, the adjunctive delivery of topical antibiotics into periodontal pockets could overcome the shortcomings of mechanical therapy (Killoy 2002, Quirynen et al. 2002). Few studies have evaluated the effectiveness of the adjunctive use of tetracycline (TE) fibres in sites that respond poorly to SRP (Flemmig et al. 1996, Vandekerckove et al. 1997, Kinane & Radvar 1999, Mombelli et al. 2002). Apart from two studies (Corsair 1994, Wilson et al. 1997), there are no investigations that have evaluated for more than 6 months follow-up clinical, radiological and microbiological benefits for combined therapy at non-responsive areas.

The aim of the present study was to compare the clinical, radiological and microbiological effects of SRP with and without TE-loaded fibres on sites with previously unsuccessful mechanical therapy 6 and 12 months post-operatively.

Materials and Methods

Subjects

The participants were 19 (11 women and eight men), aged 47.00 ± 10.78 years, consecutively selected among those attending the Periodontal Department of the University of Torino for chronic periodontitis therapy. They had previously (at least 3 months before the screening visit) received four quadrant SRP and, despite mechanical treatment and good oral hygiene (full mouth plaque score, FMPS $<20\%$), they still had pockets with bleeding on probing (BOP). Subjects were selected if they had at least four non-adjacent teeth in opposite quadrants with probing depths (PDs) between 4 and 5 mm, BOP and without furcation involvement (Listgarten et al. 1991). Pockets on third molars and incisors (apart from the distal surface of the second incisors) were also excluded to prevent bacterial contamination from the opposite quadrants (Wong et al. 1999).

Exclusion criteria included pregnancy, lactation, systemic disease that might have affected the progression or treatment of periodontitis (Heijl et al. 1991), smoking habits (Ah & Johnson 1994), antibiotic intake in the previous 3 months (Heijl et al. 1991) and allergy to TE.

Patients fulfilling the inclusion criteria were informed of the study and gave informed consent forms approved by the Ethics Committee of the Medical Faculty, University of Turin.

Treatment

Two non-adjacent teeth with persistent periodontitis were randomly selected to receive SRP plus TE-loaded fibres (test sites) and two on the opposite quadrants to receive SRP alone (control sites) according to a split-mouth design.

The four-study sites in each patients received SRP under local anaesthesia extended to all teeth of the sextants and fibres (Actisite® Alza Corporation, Palo Alto, CA, USA), and were inserted into the test pockets using a blunt instrument (Goodson 1996). A cyanoacrylate adhesive (Histoacril®, B. Braun SPA, Milan, Italy) was placed on the gingival margin of test sites to secure the fibres and on the control sites to create a comparable subgingival environment. All the treatments were performed by the same operator.

Patients were instructed to rinse with 15 ml 0.2% chlorhexidine digluconate solution twice a day for 2 min and to refrain from brushing the selected sites. If fibres were dislodged before 5 days, patients returned for their replacement. The fibres were retained 10 ± 2 days (Goodson 1996) because they maintained in the gingival crevicular fluid a mean concentration greater than 1300 μg/ml (Tonetti et al. 1990). Thereafter, rinsing was stopped and regular oral hygiene was resumed. Patients were checked monthly for home-care procedures that, if necessary, were reinforced. Subjects also received full-mouth maintenance scaling at 3, 6, 9 months post-therapy.

Measurements

Clinical measurements

Subjects were clinically monitored at baseline (immediately before the experimental treatment), 6 and 12 months post-operatively by the same examiner who was blinded to the treatment.

Plaque accumulation (PI 0/1), BOP 0/1, PD, recession (REC) and clinical attachment loss (CAL) were scored at six sites per tooth at all teeth using a William 0 probe, but statistical analysis was performed only on the values recorded at test and control sites. Clinical parameters also included FMPS and full-mouth bleeding score (FMBS).

Radiographic measurements

Standard periapical radiographs of the four sites (for sites in posterior sextants also bite-wing films) were taken at baseline and after 6 and 12 months using the Rinn alignment system with personalized Duralay bite blocks. All images were captured with a scanner and stored on a hard disk. The distance from the cemento-enamel junction (CEJ) to the bottom of the bone defect was measured with the Image-Lab software specifically written by our Radiology Department in conjunction with the Turin Polytechnic. In the absence of a detectable CEJ, the most apical margin of a restoration was used as the reference point.

Microbiological analysis

Samples were collected at baseline, at the removal of the fibres, 6 and 12 months post-therapy. Sites were isolated with cotton rolls and gently air-dried. Subgingival plaque samples were collected with a sterile paper point inserted down to the bottom of the pocket, kept in place for 20 s (Mombelli et al. 1996) and immediately placed in sterile Eppendorf vials containing 0.5 cm^3 reduced transport fluid. The vials were vortexed for 1 min and then stored at $-70^{\circ}C$ until multiplex polymerase chain reaction (PCR) and DNA probe identification of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *B. forsythus* and *T. denticola* (Parodontosi®, Bioline, Amplimedical SPA, Assago, Italy) by an examiner blinded to the treatment applied. The specificity of primers was tested by amplification of pure cultures of bacterial control strains (ATCC no. 33277 *P. gingivalis*, 43037 *A. actinomycetemcomitans*, 33520 *T. denticola*, 43037 *B. forsythus* and 25611 *P. intermedia*). The sensitivity of this qualitative assay is 100 cells/site of each species (Caposio et al. 2003).

Statistics

The outcome variables evaluated in this study were the changes in the average clinical, radiological and microbiologi-

cal parameters from pre- to various times post-therapy.

A paired *t*-test was used to analyse the baseline, 6- and 12-month PD, REC, CAL and bone level mean values.

Unpaired Student's *t*-test and one-way ANOVA were employed to analyse the differences between the test and control sites at the three time points and gave the same *p*-values. The χ^2-test was used to analyse the PI and BOP values between treatment groups and within each group. The numbers and percentages of sites free of periodontal pathogens and prevalences of the five pathogens were calculated at baseline, immediately after treatment and after 6 and 12 months, and analysed with the non-parametric *z*-test. The significance cut-off was $p<0.05$.

Results

Clinical and radiological

All patients completed the study. Each test tooth received a mean TE HCl dosage of about 3.25 ± 0.73 mg (6.26 ± 1.4 cm fibre length) and the fibres were in place for an average of 10 ± 0.75 days. At baseline, no significant differences between TE and SRP groups were found in any of the parameters assessed ($p>0.41$, Table 1).

Table 2 presents the clinical variables recorded at 6 and 12 months.

The study population maintained relatively good oral hygiene and the PI scores did not differ significantly among the treatment groups at 6 and 12 months ($p \geqslant 0.753$). The percentage of BOP sites decreased significantly ($p<0.0001$) in both groups (Fig. 1) from 100% at baseline to 47.4% and to 57.9% at 6 and 12 months, respectively, in the SRP group and from 100% to 13.15% and 23.7% in the test group. The greatest reduction occurred in the test teeth compared with the control group for the 6 ($p=0.0027$)- and 12-month ($p=0.005$) follow-up. The difference between the 6- and 12-month mean values within each group was not significant ($p=0.3$ test group and $p=0.49$ control group).

In both groups, a significant PD reduction and CAL gain ($p \leqslant 0.004$) at 6 and 12 months post-therapy were observed. After 6 months, PD reduction and gain in clinical attachment amounted to 1.39 ± 0.92 and 1.16 ± 0.95 mm, respectively, in the control group compared with 2.21 ± 0.66 and $2.03 \pm$

Table 1. Comparison between treatment groups at baseline

Treatment group	PI	BOP	PD	CAL	REC
SRP+T. fibres (test teeth)	18.4%	100%	4.85 ± 0.39	5.71 ± 1.23	0.89 ± 1.13
SRP alone (control teeth)	21%	100%	4.74 ± 0.45	5.79 ± 1.12	1.05 ± 0.93
difference between treatments	$p=1.00$	/	$p=0.41$	$p=0.76$	$p=0.50$

BOP = bleeding on probing, PD = probing depth, CAL = clinical attachment loss.
PI-Pllaque accumulation or plaque Index

Table 2. Overall clinical results

Clinical parameters and statistical significance of each therapy	SRP+T. fibres (test teeth)	SRP alone (control teeth)	Difference between treatments
PI at 6 months	10.5%	7.9%	$p=1.00$
PI at 12 months	15.8%	15.8%	$p=0.753$
χ^2 between 0 and 6 months	$p=0.51$	$p=0.192$	
χ^2 between 0 and 12 months	$p=1.00$	$p=0.767$	
BOP at 6 months	13.15%	47.4%	$p=0.0027$
BOP at 12 months	23.7%	57.9%	$p=0.005$
χ^2 between 0 and 6 months	$p<0.00001$	$p<0.00001$	
χ^2 between 0 and 12 months	$p<0.00001$	$p=0.00002$	
PD at 6 months	2.61 ± 0.64	3.26 ± 0.92	$p=0.0006$
PD at 12 months	2.79 ± 0.96	3.53 ± 1.2	$p=0.004$
paired *t*-test between 0 and 6 months	$p<0.00001$	$p<0.00001$	
paired *t*-test between 0 and 12 months	$p<0.00001$	$p<0.00001$	
CAL at 6 months	3.66 ± 1.3	4.63 ± 1.5	$p=0.0035$
CAL at 12 months	3.95 ± 1.63	5.26 ± 1.33	$p=0.00025$
paired *t*-test between 0 and 6 months	$p<0.00001$	$p<0.00001$	
paired *t*-test between 0 and 12 months	$p<0.00001$	$p=0.0033$	
REC at 6 months	1.05 ± 1.14	1.37 ± 1.17	$p=0.23$
REC at 12 months	1.16 ± 1.22	1.76 ± 1.32	$p=0.043$
paired *t*-test between 0 and 6 months	$p=0.031$	$p=0.00019$	
paired *t*-test between 0 and 12 months	$p=0.0058$	$p=0.00001$	

BOP = bleeding on probing, PD = probing depth, CAL = clinical attachment loss.
PI-Pllaque accumulation or plaque Index

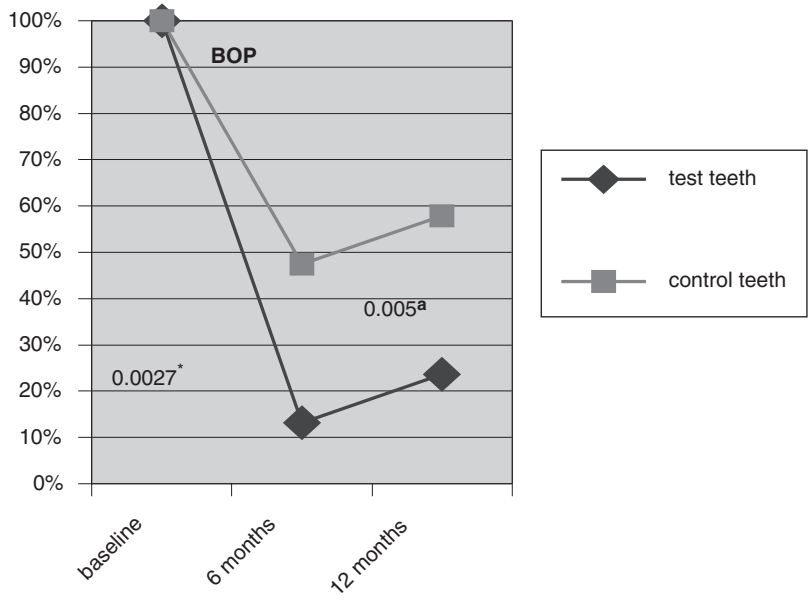

Fig. 1. Percentage of sites bleeding on probing (BOP). *p-value for differences between test (◆) and control (■) teeth at 6 months; [a]p-value for differences between test and control teeth at 12 months.

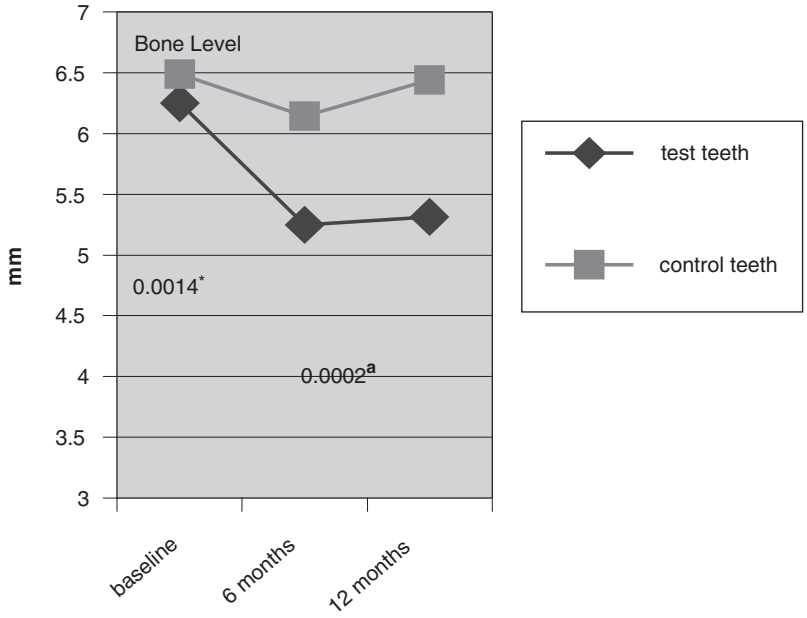

Fig. 2. Bone level. *p-value for differences between test (◆) and control teeth (■) at 6 and 12 months; [a]p-value for differences between test and control teeth at 12 months.

Table 3. Sites free of pathogens (number and percentage)

Treatment group	Baseline	Post-therapy	6 months	12 months
SRP+ T. fibres (test teeth)	1 (2.6%)	28 (73.7%)	24 (63.2%)	21 (55.5%)
SRP alone (control teeth)	0 (0%)	4 (10%)	4 (10%)	5 (13.2%)
difference between treatments	$p = 0.158$	$p = 0.0001$	$p = 0.0001$	$p = 0.0005$

Table 4. Prevalence of single species (site number)

	Baseline	Post-therapy	6 months	12 months
SRP+T. fibres (test teeth)				
Actinobacillus actinomycetemcomitans	26	0	7	9
Prevotella gingivalis	28	10	10	10
P. intermedia	10	0	2	3
Bacteroides forsythus	14	3	3	3
Treponema denticola	16	5	7	2
SRP alone (control teeth)				
A. actinomycetemcomitans	28	16	14	19
P. gingivalis	29	29	24	21
P. intermedia	12	10	12	7
B. forsythus	19	7	10	9
T. denticola	19	10	9	5

0.79 mm in the TE group. Whereas the PD reduction and attachment level gain could be nearly stabilized in the test group after 1 year (PD 2.05 ± 0.93, $p = 0.128$; CAL 1.71 ± 1.14, $p = 0.069$), a rebound was observed in the control group with a mean PD difference of 1.21 ± 1.12 ($p = 0.039$) and attachment gain of only 0.53 ± 1.03 mm ($p = 0.0001$) after 12 months. The differences in CAL and PD changes between treatment modalities were statistically significant at each time point ($p_{PD} = 0.0006$ and $p_{CAL} = 0.0035$ at 6 months, $p_{PD} = 0.004$ and $p_{CAL} = 0.00025$ at 12 months). In the course of healing, a significant increase ($p \leqslant 0.031$) in recession depth was noticed in both groups compared with baseline, but the differences between REC changes in the treatment groups were significant only at 12 months ($p = 0.043$). REC mean change in the TE group was -0.26 ± 0.55 mm as compared with -0.66 ± 0.81 mm in the SRP group.

The bone level showed a significant gain after 6 months in both groups ($p = 0.0001$). In the fibres treated defects the level increased for 1.02 ± 0.44 mm from 6.25 ± 1.18 to 5.25 ± 1.14 mm, in the control defects the gain was 0.35 ± 0.5 mm from 6.49 ± 1.27 to 6.14 ± 1.2 mm. After 12 months, a rebound was observed in the control group to 6.44 ± 1.21 mm with a gain of 0.09 ± 0.72 mm compared with baseline ($p = 0.389$), whereas in the test group the bone level showed a small change to 5.31 ± 1.33 mm with a gain of 0.94 ± 0.68 mm. The differences between the test and control groups were significant at 6 and 12 months (Fig. 2).

Microbiological observations

Change in the prevalence of bacterial species

A total of 76 plaque samples were analysed from 19 subjects at baseline, at removal of fibres, at 6 and 12 months. Table 3 shows the number of sites that harboured the target species at each time point. In one site test no bacterial species was detected prior to treatment. The application of TE decreased the number of sites positive for monitored species more than SRP alone. Twenty-eight test sites at the removal of fibres, 24 at 6 months and 21 at 12 months, were free of pathogens unlike 4, 4 and 5 control sites at the same time points ($p =$ 0.0001, 0.0001 and 0.0005, respectively).

Table 4 shows the prevalence of the bacteria recovered from the sampled sites. Prior to treatment, *P. gingivalis* and *A. actinomycetemcomitans* were detected in 75% and 71%, respectively, and *T. denticola* and *B. forsythus* in 43% and 46% of the total sampled sites. The least prevalent species, *P. intermedia*, was recovered from only 29% of the total plaque samples. There were no statistically significant differences between the two treatments groups with respect to relative bacterial colonization.

The pattern of change of the monitored bacteria after combined therapy or SRP alone differed for each species. At fibres removal, neither *A. actinomycetemcomitans* nor *P. intermedia* could be detected in the fibre-treated sites, but they were found again at the 6- and 12-month periods. In the control teeth, indeed, *A. actinomycetemcomitans* decreased to 42.1% at the 10-day examination and increased at 12 months to

50%. The number of *P. intermedia*-positive control sites remained high throughout the study. In the case of *P. gingivalis*, the proportion decreased to 26.3% in the TE-treated group at 10 days post-treatment examination and no further increase was observed after 6 and 12 months. In the control group, the prevalence of *P. gingivalis* showed a small decrease at 6 and 12 months. The proportions of *B. forsythus* decreased to 7.9% in the test sites and to 18.4% in the control teeth and remained similar during the subsequent year. Furthermore, statistical analysis of the data indicated that the combined therapy was more efficient than SRP alone to achieve a lower prevalence of *A. actinomycetemcomitans* ($p \leqslant 0.018$), *P. intermedia* ($p \leqslant 0.04$), *P. gingivalis* ($p \leqslant 0.0025$) and *B. forsythus* ($p \leqslant 0.04$) at various time points. However, the proportions of *T. denticola* were not significantly lower in test sites than in the scaling group at any interval.

Discussion

The results of the present study demonstrated that adjunctive topical application of TE-loaded fibres improves the periodontal health of patients with localized persistent periodontitis, despite previous mechanical therapy, for as long as 12 months after treatment. Compared with SRP alone, the use of TE fibres resulted in significantly greater improvements in BOP, PD and clinical attachment level. The mean PD reduction (2.05 mm at 12 months) in the TE group was consistent with the range of values reported by Hung & Douglass (2002) in meta-analysis (range: PD 1.00–2.80 mm; range: CAL 0.66–2.34 mm). However, the present results were better than those published by Newman et al. (1994) (1.81 mm) and Flemmig et al. (1996), and they were lower than those reported by Vandekerckhove et al. (2.60 mm). This discrepancy is probably due to the differences in defect selection and in the study design. In the present investigation, measurements were performed on moderate sites while in those of Flemmig et al. and of Vandekerckhove et al. only deep pockets (mean PD 6 and 6.8 mm, respectively) were selected. Furthermore, in the latter research all residual pathological pockets were treated with fibres in a full-mouth approach and in the Flemmig investigation, scaling was not repeated before fibre insertion.

Furthermore, it should be noted that more than half the sites in the control group were BOP positive with a partial CAL relapse after 12 months, whereas improvement in the test sites remained substantially stable. In the test sites, PD reduction was almost completely related to an increase in attachment level, whose 12-month stability could prove that fibres in conjunction with SRP reduce the need for surgery more than SRP alone.

Alveolar bone remineralization may be triggered by the elimination of infection. The effects of SRP with and without fibres were therefore compared radiographically. No change in the bone level was found in the control sites at 12 months, accordingly with a persistent inflammation, whereas the test sites displayed a 0.9 mm gain in bone height. The clinical and radiographic changes were thus aligned and maintained throughout the study period.

The efficacy of periodontal therapy is directly related to the ability of the treatment to lower the level and the prevalence of one or more pathogenic species. The high prevalence at baseline of the monitored bacteria suggests that they play a role in non-responsive sites in agreement with Edwardsson et al. (1999), who have considered *P. gingivalis*, *P. intermedia* and *B. forsythus* a causal factor in therapy-resistant periodontitis.

Several studies have well documented (Maiden et al. 1991, Sanz et al. 1997) the short-term effects of TE on the subgingival microflora and have concluded that TE fibres and SRP could reduce significantly the number of detectable species as compared with baseline in chronic adult periodontitis. However, the long-term effects of combined therapy are uncertain (Lowenguth et al. 1995, Wong et al. 1999, Mombelli et al. 2002). In the present study we chose the PCR technique to detect bacteria. There were three studies (Goodson et al. 1991, Maiden et al. 1991, Lowenguth et al. 1995) that used a DNA probe to determine the microbiological changes after the local use of TE, but they did not analyse patients with persistent periodontal pockets and in the Maiden et al. investigation (1991), no data were available for the effect on *B. forsythus.* Data of the literature report that TE fibres are suitable for topical application because they reach a high concentration in the gingival fluid (1300 μg/ml), and there is a moderate resistance of periodontal bacteria against the drug. The range of minimal inhibitory concentrations for most periodontopathic bacteria varies from 0.1 to 128 μg/ml (Pajukanta et al. 1993) and in patients with refractory periodontitis TE resistance was predominantly found in bacteria not considered periodontal pathogens (Olsvik et al. 1995).

In the present study, the percentage of positive sites for monitored species decreased immediately after treatment in the fibre group (26%), whereas in the SPR group it remained high (89%). The pattern of change after combined therapy and SRP alone differed for each bacterial species. The combined therapy was more effective than the mechanical treatment in reducing the prevalence of *P. gingivalis*, *P. intermedia*, *B. forsythus* and *A. actinomycetemcomitans*, whereas there was no difference between the two therapies in the prevalence of *T. denticola*.

At test sites, *A. actinomycetemcomitans* could not be detected 10 days following treatment, but recolonized 18% and 23% of sites at 6- and 12-month periods.

This pattern might be due to tissue invasion, which may protect bacteria from the fibre therapy or to the recontamination from the cheek, the mucosa tongue and the extracrevicular sites. These findings are in agreement with those of Maiden et al. (1991) and Wong et al. (1999) and Mombelli et al. (2002). The TE was unable to eliminate completely the black-pigmented anaerobes *P. gingivalis* and *P. intermedia* as already reported by Mombelli et al. (1996, 2002) and Lowenguth et al. (1995). In the control group, SRP appeared to have a modest effect on the composition of subgingival microbiota. Only three species, *A. actinomycetemcomitans*, *B. forsythus* and *T. denticola*, were significantly decreased and none of them or any other bacteria were detectable post-therapy. These data confirm the decrease in the prevalence and levels *of T. denticola* and *B. forsythus* reported in other studies (Lowenguth et al. 1995, Haffajee et al. 1997a). Further, in agreement with other investigations (Mombelli et al. 1994, Haffajee et al. 1997a), black-pigmenting anaerobes were not decreased but they had a spontaneous decline between 6 and 12 months. This pattern might be

explained by means of the effects of SRP on the host–parasite equilibrium (Haffajee et al. 1997a).

Microbiological changes achieved by SRP in this investigation were greater than those of other studies, which reported a return within a short period of time of the total number of bacteria to the pretherapy levels (Haffajee et al. 1997a, Cugini et al. 2000). Because the present study is a split-mouth design it is possible that the TE may have a crossover effect to control sites. It has been shown that following placement of fibres, the drug is transiently detected in the serum or saliva, which may influence our microbiological results.

In conclusion, clinical, radiological and microbiological benefits were obtained from the adjunctive TE fibres at non-responsive sites. The fact that 44.74% of the test sites harboured one or more species at 12 months did not worsen the clinical and radiological results. Sites that harboured bacteria in excess of the very low multiplex PCR and DNA probe identification threshold were classed as ''positive'', and may not have contained enough bacteria to have a pathogenic effect in an environment now favourable to the host (Gunaratam et al. 1992).

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