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Microbiological outcomes of quadrant *versus* full-mouth root planing as monitored by real-time PCR

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Abstract

Objectives: To study the short-term microbiological changes following full-mouth compared with quadrant wise scaling and root planing (FMRP and QRP) as well as long-term effects.

Method: Twenty patients with chronic periodontitis were randomized into a test group treated in two sessions with subgingival scaling and root planing within 24 h (FMRP) and a control group treated quadrant by quadrant in four sessions at intervals of one week (QRP). Microbiological samples were taken in the two deepest pockets of the maxillary right quadrant immediately before treatment and after 1 day, 1, 2, 4, 8, 12, and 24 weeks. The samples were evaluated by real-time PCR for quantification of *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* ssp., *Porphyromonas gingivalis*, *Prevotella intermedia, Treponema denticola*, and *Tannerella forsythia* as well as for total bacterial counts (TBC).

Results: Treatment resulted in a TBC median log reduction of 0.75 (FMRP) and 0.72 (QRP). There were no differences between groups either for the short term (1 day–4 weeks) (analysis of variance: $p = 0.3150$) or for long term (4–24 weeks) (analysis of variance: $p = 0.9671$). Likewise, no differences were detected for selected target bacteria.

Conclusion: The results of the present study showed similar microbiological outcomes following both treatment modalities.

Key words: chronic periodontitis; microbiological changes; periodontal pathogens; periodontal therapy; re-colonization; scaling and root planing

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Deep pockets, grooves, furcations, and concavities on the root surface can complicate sufficient debridement of such infected sites with periodontal instruments (Heitz-Mayfield et al. 2002, Umeda et al. 2004). Incomplete elimination of periodontal pathogens by non-surgical therapy might result in relatively rapid re-colonization and recurrence of the disease (Umeda et al. 2004). The impact of a single course of scaling and root planing on *Porphyromonas gingivalis*, *Prevotella intermedia*, spirochetes, and motile rods using cultivation and phase-contrast microscopy for identification had been investigated earlier (van Winkelhoff et al. 1987). The results showed that the total numbers of spirochetes and motile rods in the sample did not change significantly during the 8 weeks. However, black-pigmented *Bacteroides* species were found not only subgingivally in the periodontal pocket, but also at various sites in the oral cavity such as the tonsils, dorsum of the tongue, and saliva (Zambon et al. 1981, van Winkelhoff et al. 1988). The findings implied that when *P. gingivalis* is present in the saliva of patients with suppurative periodontitis, there is a possibility of contamination of other oral sites (van Winkelhoff et al. 1988).

Based on these reports, the treatment strategy of one-stage full-mouth disinfection was developed, to prevent bacterial re-colonization originating from intra-oral niches such as untreated pockets, tonsils, dorsum of the tongue (Quirynen et al. 1995).

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interest.

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A series of studies compared the clinical and microbiological effects of scaling and root planing within 24 h, supplemented with supra- and subgingival use of chlorhexidine, with partial-mouth debridement (Mongardini et al. 1999, Quirynen et al. 1999, 2000). Only minor differences were found between full-mouth disinfection using chlorhexidine and full-mouth root planing; both full-mouth treatment modalities showed a greater impact on the subgingival microbiota than quadrantwise scaling and root planing (QRP). For microbiological analysis, differential phase-contrast microscopy and analysis with bacterial cultivation was used. De Soete et al. (2001) analysed bacterial samples from the studies of Mongardini et al. (1999) and Quirynen et al. (1999) with checkerboard DNA–DNA hybridization. Full-mouth disinfection showed additional improvements compared with conventional treatment. The authors of these series of clinical and microbiological studies advocated the adoption of the idea of a delay of cross-contamination or intra-oral translocation through a one-stage, full-mouth approach (Quirynen et al. 2006).

Apatzidou et al. (2004) tested the hypothesis of an enhanced impact on periodontopathogenic bacteria by full-mouth root planing in comparison with quadrantwise treatment. Both therapies resulted in marked reductions of target bacteria, but no differences between the two groups could be demonstrated 6 months post-scaling. Koshy et al. (2005) investigated the effects of quadrantwise *versus* single-visit full-mouth ultrasonic debridement with or without the use of an antiseptic. No significant differences between treatment modalities with respect to microbiological data could be found after 6 months. Both Apatzidou et al. (2004) and Koshy et al. (2005) used PCR for bacterial identification. However, none of the above-mentioned studies have investigated early microbiological changes in treated sites in an oral cavity with remaining and yet untreated periodontal pockets.

Thus, the aim of the present study was to determine early microbiological changes after 1 day, 1, 2, and 4 weeks following full-mouth compared with quadrantwise root planing as well as long-term microbiological effects after 8, 12, and 24 weeks.

Material and Methods

Clinical study design and treatment modalities have already been described in a preceding article (Jervøe-Storm et al. 2006); briefly, the study/treatment protocol was as follows:

Patients

A total of 20 adult patients (all caucasians, 53.1 years ± 10.2 of age, nine females, and 11 males) with untreated chronic periodontitis volunteered in the present study. Patients were not included if their systemic health precluded periodontal treatment or if they were pregnant. Patients had received no subgingival scaling and root planing previously and had taken no antibiotics within the preceding 6 months. Patients had more than 20 teeth, with at least two teeth per quadrant with a probing depth of 5 mm or more and bleeding on probing (BOP). Patients were considered as smokers if they were smoking more than 10 cigarettes a day for more than 5 years (Kinane & Radvar 1997, Quirynen et al. 2000). Before baseline, each patient was given repeated oral hygiene training until a low plaque score was obtained. Throughout the study period, oral hygiene was repeatedly reinforced after 3, 4, 5 and 6 months to assure low plaque levels.

Study design

All patients who fulfilled the inclusion criteria and agreed to participate in the study signed an informed consent. The study had been approved by an international ethics committee (IRB/IEC, Freiburg, Germany).

At a baseline visit, clinical measurements were performed. Patients were randomized into two groups according to a computer-generated list provided by an external agent. The patient characteristics are presented in Table 1.

Clinical parameters

Clinical parameters were assessed before the first session of scaling and root planing and after 3 and 6 months. All measurements were performed by one blinded and calibrated examiner.

Full-mouth probing pocket depths (PPDs) were recorded with a computerized constant force probe (Florida Probe®, Gainesville, FL, USA) at six sites per tooth. Using an individual stent, the relative attachment level (RAL) was also recorded at the same six sites per tooth. Both PPD and RAL were measured to the nearest whole millimetre. BOP was recorded concomitantly with PPD and RAL.

Sampling sites

The two deepest pockets from two different teeth of the right maxillary quadrant with PPD ⩾5 mm, BOP, and no furcation involvement were selected as sampling sites. The selection of the sampling sites was made before baseline; subsequent samples were taken at the same sites. In both groups, microbiological samples were collected in a standardized way by the same blinded investigator. Subgingival plaque samples were obtained at baseline immediately before instrumentation, after 1 day, 1, 2, 4, 8, 12, and 24 weeks with two simultaneously inserted sterile paper points for 20 s that were immediately transferred into a sterile transport tube. Samples were sent to a specialized laboratory (Carpegen GmbH, Münster, Germany) for evaluation by real-time PCR (meridol® Perio Diagnostics, GABA International, Münchenstein, Switzerland) for detection and quantification of *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* ssp., *Porphyromonas gingivalis*, *Prevotella intermedia, Treponema denticola*, and *Tannerella forsythia* as well as total bacterial counts (TBC), as described by Jervøe-Storm et al. (2005). The level of detection was set to 10^3 bacteria/plaque sample.

Table 1. Patient characteristics at baseline (mean ± SD)

	QRP ($n = 10$)	FMRP ($n = 10$)	p - value t - test
Age	49.1 ± 8.6	56.3 ± 11.1	0.167
Male/female	4/6	5/5	0.673
Smoker	1	1	1.000
Plaque index (O'Leary)	16.1 ± 8.8%	14.9 ± 6.8%	0.357
BOP	22.1 ± 12.4%	16.9 ± 13.7%	0.385
PPD of sampling sites	6.4 ± 0.85 mm	5.9 ± 0.91 mm	0.222
BOP of sampling sites	85 ± 24.2%	70 ± 25.8%	0.196

QRP, quadrant wise scaling and root planing (control group); FMRP, full-mouth scaling and root planing (test group); BOP, bleeding on probing; PPD, probing pocket depth.

The bacterial plaque samples were numbered consecutively; the microbiological laboratory was blinded. All clinical and microbiological findings were given to an independent person, who solely could decode all results.

Treatment

In the test group (FMRP), 10 patients were treated in 2 sessions with subgingival scaling and root planing within 24 h on 2 consecutive days, starting with the right maxillary and mandibular quadrants. In the control group QRP, 10 patients were treated with subgingival scaling and root planing, quadrant by quadrant clockwise in four sessions at intervals of 1 week. Treatment always started in the right maxillary quadrant in both groups to facilitate the comparison between the groups. Scaling and root planing was performed under local anaesthesia using periodontal hand instruments (Gracey curettes; Hu-Friedy, Chicago, IL, USA) and sonic scalers (Sonicflex; KaVo, Biberach, Germany). Instrumentation in both groups was performed without use of any antibiotics or antiseptics. All oral hygiene instructions and the subgingival debridement were performed by the same therapist. The duration of scaling and root planing was about 1 h per quadrant in both groups, so that the total time spent for each patient averaged 4 h, independent of group affiliation.

Statistical analysis

Before analysis of the TBC and all six target bacteria over time, bacterial counts were transformed to logarithms (base 10). In order to avoid problems with 0 counts of bacteria, 1 was added to each value for quantitative analysis. After transformation, the mean of the two values from the two sites per patient and visit was taken for further analysis. Repeated measurement analyses of variance, with the factors ''time'', ''treatment group'', and ''time × treatment interaction'' calculated, were performed for each count value with the time points 1 day, 1, 2, and 4 weeks for the short-term effects and for the time points 4, 8, 12, and 24 weeks for the long-term effects. The significance level was set at $p < 0.05$.

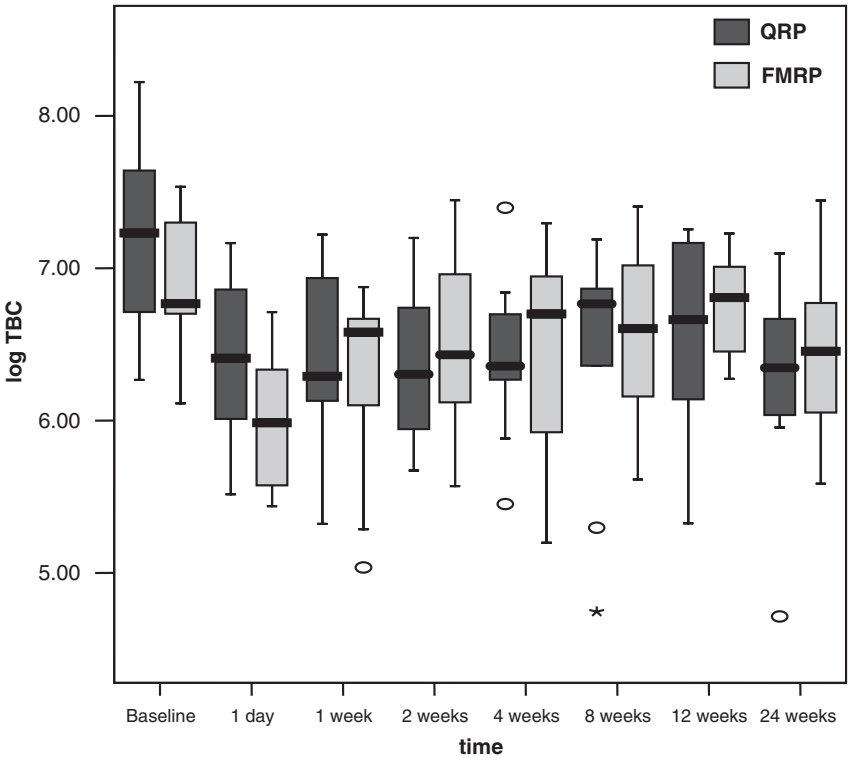

Fig. 1. Effect over time of quadrant wise scaling and root planing (QRP) *versus* full-mouth scaling and root planing (FMRP) on the total bacterial counts (TBC) (log bacteria/plaque sample). Box-plot shows median, inter-quartile range between the 25th and 75th percentile; whiskers indicate maximum and minimum; and outliers are shown as circles and asterisks, $n = 10$ in each group.

Results

Microbiological results

Total Bacterial Counts (TBC)

In both groups, treatment resulted in a marked reduction of TBC at day 1 (median log reduction FMRP: 0.75; QRP: 0.72). The findings from day 1 were used as the starting point for evaluation of the microbiological changes associated with the two treatment modalities. Figure 1 illustrates the changes in TBC for both groups over time. During the first 4 weeks (short-term effects), only slight changes in the median values in the QRP group could be noticed; in the FMRP group, an increase in the median values for TBC from the first day to 4 weeks was found. No major difference in median TBC could be observed in both groups at 4 and 24 weeks (long-term effects). Statistical testing indicated no differences between both groups, either for short (analysis of variance: $p = 0.3150$) or for long-term effects (analysis of variance: $p = 0.9671$).

A. actinomycetemcomitans

A. actinomycetemcomitans was only detected in a small number of sites and patients. The relative proportions (as percentage of TBC) as well as the number of *A. actinomycetemcomitans*-positive patients in the two groups are presented in Table 2. Figure 2 presents the log numbers for each of the *A. actinomycetemcomitans*-positive sites (adapted from Mombelli et al. 1995). The quadrantwise treatment led to one of five *A. actinomycetemcomitans*-positive patients at 24 weeks (20%), whereas three of five patients in the FMRP group were still positive (60%). No difference was found between groups, either for short (analysis of variance: $p = 0.4926$) or for long-term (analysis of variance: $p = 0.3882$) effects.

F. nucleatum ssp.

No differences in the frequency of detection and relative proportions were found for *F. nucleatum* ssp. (Table 2). At baseline, the level of *F. nucleatum* ssp. was higher in six sites in the QRP

group. Both groups showed only a slight difference in the changes occurring during the 24 weeks (Fig. 2).

No difference between treatment groups was found either in the short term (analysis of variance: $p = 0.6815$) or in the long term (analysis of variance: $p = 0.1847$).

P. gingivalis

The relative proportions of *P. gingivalis* were relatively high at baseline (11% respectively 9%). Both treatments reduced the proportions of *P. gingivalis*; however, baseline values were almost reached after 24 weeks (Table 2). Starting with day 1, only small differences regarding the frequency of detection of *P. gingivalis* were found between groups (Fig. 2). No statistical difference between treatment groups was found either in the short term (analysis of variance: $p = 0.1265$) or in the long term (analysis of variance: $p = 0.8289$).

P. intermedia

As a result of therapy, the relative proportions of *P. intermedia* were reduced at day 1 (Table 2). However, in the FMRP group, a more pronounced increase in the relative proportions was seen within the first 4 weeks. After 24 weeks, the relative proportions were close to baseline levels in both groups (Table 2). Even though the frequency of detection was higher in the QRP group, the number of sites with the order of counts of five and six were almost similar throughout the study period (Fig. 2). No statistical difference between the treatment groups was observed either in the short term (analysis of variance: $p = 0.7969$) or the long term (analysis of variance: $p = 0.5335$).

T. denticola

No differences in the frequency of patients positive for *T. denticola* and relative proportions at 4 and 24 weeks were found (Table 2). The microbiological changes within both groups were comparable (Fig. 2). No statistical difference was seen between the treatment groups either in the short term (analysis of variance: $p = 0.1587$) or the long term (analysis of variance: $p = 0.1394$).

T. forsythia

In both groups, a similar reduction in the frequency of patients positive for *T. forsythia* was found (Table 2). For both groups, similar relative proportions of *T. forsythia* were found at most of the evaluation time points (Table 2). Likewise, the log numbers of positive sites in both groups were comparable during the study period (Fig. 2). Statistical testing indicated no difference between both group, for short-term effects (analysis of variance: $p = 0.2291$). For long-term effects, a significant difference was found (analysis of variance: $p = 0.0270$), with less favourable results in the QRP group.

Clinical results of the sampling sites

An improvement of clinical parameters could be achieved in both groups during the 6-month study (Table 3), in concordance with the clinical findings of the whole-mouth recordings and the results of the maxillary right quadrant, which have been previously reported (Jervøe-Storm et al. 2006).

Discussion

The aim of this study was to compare the short-term microbiological changes following either quadrantwise or full-mouth scaling and root planing (FMRP), because previous studies had suggested that untreated periodontal pockets could be sources of bacterial re-infection.

In both groups, treatment resulted in a marked reduction of TBC at day 1. In the present study, both treatment modalities resulted in less *A. actinomycetemcomitans*-positive patients at day 1. The quadrantwise treatment resulted in a marked reduction of *A. actinomycetemcomitans*-positive patients at 24 weeks compared with the FMRP group. How-

Table 2. Relative proportions, in percentage of TBC (total bacterial counts), in positive sites for the presence of specific bacteria in the two treatment groups (frequency of patients positive for the presence of specific bacteria)

	Baseline	First day	First week	Second week	Fourth week	Eight week	Twelfth week	Twenty fourth week
A.a.								
QRP	0.52 (3)	1.69 (2)	0.28 (2)	0.21 (2)	0.29 (1)	0.62 (1)	0.09 (1)	0.03 (1)
FMRP	2.45 (4)	0.99 (2)	1.41 (2)	0.27 (3)	2.80 (3)	3.19 (3)	3.39 (4)	1.93 (3)
F.n.								
QRP	2.25 (10)	3.86 (6)	2.74 (9)	3.12 (7)	2.83 (8)	2.26 (8)	1.49 (8)	1.75 (7)
FMRP	1.79 (10)	2.09 (8)	1.84 (8)	5.17 (9)	1.62 (8)	1.45 (6)	2.11 (9)	0.94 (8)
P.g.								
QRP	10.95 (10)	2.42 (10)	9.96 (7)	5.81 (8)	5.61 (9)	5.01 (7)	1.42 (10)	8.02 (8)
FMRP	9.30 (8)	6.07 (7)	3.68 (8)	4.64 (8)	7.13 (7)	1.52 (7)	4.49 (9)	7.47 (8)
P.i.								
QRP	2.86 (7)	1.20 (7)	2.51 (6)	3.31 (7)	3.41 (5)	1.90 (7)	4.30 (6)	2.25 (6)
FMRP	2.22 (5)	1.71 (2)	6.35 (4)	14.83 (3)	6.81 (4)	4.07 (4)	0.61 (5)	3.85 (3)
T.d.								
QRP	5.77 (10)	3.94 (7)	6.84 (6)	5.99 (7)	4.99 (9)	4.91 (8)	3.77 (8)	5.35 (8)
FMRP	7.31 (9)	4.41 (9)	2.32 (8)	3.45 (8)	4.53 (8)	1.43 (7)	8.99 (9)	5.58 (8)
T.f.								
QRP	3.35 (10)	2.04 (9)	4.09 (6)	3.38 (7)	2.03 (7)	1.96 (8)	1.29 (10)	3.19 (8)
FMRP	1.87 (10)	8.36 (9)	3.11 (7)	2.21 (8)	2.16 (8)	0.89 (7)	1.66 (9)	2.66 (8)

The patient was considered as positive when at least one sampling site was positive. Level of detection: 10^3 bacteria/plaque sample.
QRP, quadrant wise scaling and root planing (control group); FMRP, full-mouth scaling and root planing (test group). *A.a*, *Actinobacillus actinomycetemcomitans*; *F.n*, *Fusobacterium nucleatum*; *P.g*, *Porphyromonas gingivalis*; *P.i*, *Prevotella intermedia*; *T.d*, *Treponema denticola*; *T.f*, *Tannerella forsythia*.

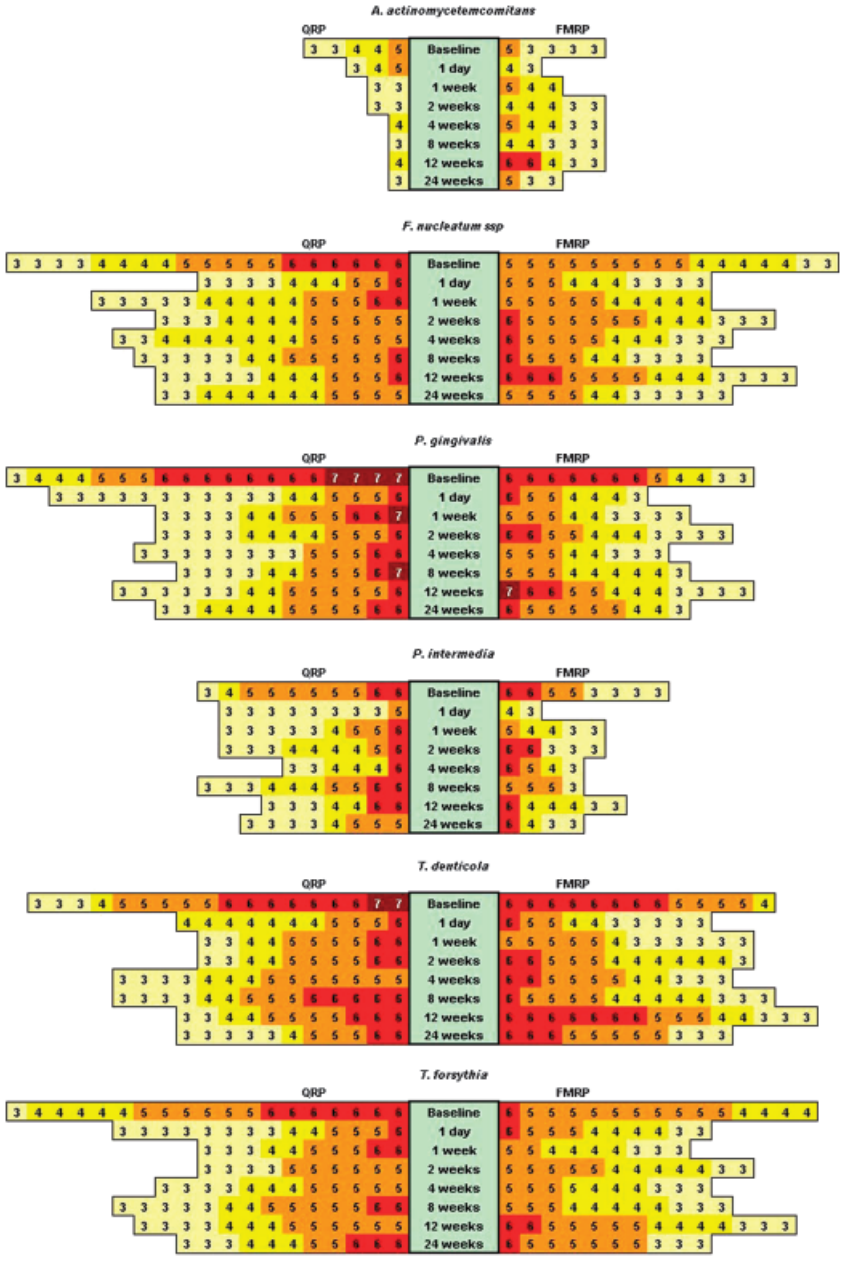

Fig. 2. Frequency of detection of six periodontal pathogens (*A. actinomycetemcomitans*, *F. nucleatum* ssp., *P. gingivalis, P. intermedia*, *T. denticola*, and *T. forsythia*) in subgingival plaque samples for quadrant wise scaling and root planing (QRP; $n = 20$) and full-mouth scaling and root planing (FMRP) groups ($n = 20$) at baseline after 1 day, 1, 2, 4, 8, 12, and 24 weeks. Each number represents one positive sample. Each patient had two sample sites. Numbers 3–7 indicate the order of bacterial counts: 3 indicating a range $\geq 10^3$ but $< 10^4$ bacteria/plaque sample, 4 indicating a range $\geq 10^4$ but $< 10^5$ bacteria/plaque sample, 5 indicating a range $\geq 10^5$ but $< 10^6$ bacteria/plaque sample, 6 indicating a range $\geq 10^6$ but $< 10^7$ bacteria/plaque sample, and 7 indicating a range $\geq 10^7$ bacteria/plaque sample (data presentation adapted from Mombelli et al. 1995). The data are not linked to any specific site.

ever, no firm conclusions could be drawn, because of the small numbers of sites and patients positive for *A. actinomycetemcomitans*. Haffajee et al. (1997) treated patients with quadrantwise scaling and root planing (QRP) and found a significant decrease in the mean prevalence and levels of *P. gingivalis, T. denticola*, and *T. forsythia* but no complete elimination. For microbial analysis, checkerboard DNA–DNA hybridization was used. Both treatment modalities in the present study obtained mean log reductions of all six target bacteria, confirming their results. Haffajee et al. (1997) concluded that scaling and root planing did not induce major changes in subgingival microbiota. Microorganisms such as *Actinomyces viscosus* and *Streptococcus sanguis* increased from baseline to 3, 6, and 9 months. This could explain the moderate increase in TBC from day 1 seen in the present study. Darby et al. (2001), using PCR found significant reductions on a site basis, but not of the percentages of positive patients for each investigated microorganism in the frequency of detection of *P. intermedia*, *T. denticola*, and *T. forsythia* after quadrant wise scaling and root planing. The present study found, in agreement with their findings, mean count reductions of *P. intermedia*, *T. denticola*, and *T. forsythia*, but no major changes in the frequency of positive patients.

At baseline, *F. nucleatum* ssp. (no 6 values in the FMRP group), *P. gingivalis* (no 7 values in the FMRP group), *T. denticola* (no 7 values in the FMRP group), and *T. forsythia* (more often six values in the QRP group) showed a higher bacterial burden in the QRP group. It can be speculated that these findings were caused by the higher mean PPD of the sampling sites in the QRP group. This might be the case for *F. nucleatum* ssp., *P. gingivalis*, and *T. forsythia*. However, 1 day after treatment, these differences were negligible. This was also the reason for evaluating the short- and long-term results on the basis of first-day findings. Thus, a more comparable basis for evaluation of both treatment groups was provided. Although patients were slightly older in the FMRP group and a tendency towards higher probing pocket depths and BOP in sampling sites were observed in the QRP group, no statistical differences were noticed between the two populations.

Only a few studies have dealt with early microbiological events after subgingival instrumentation. Mousquès et al. (1980) used darkfield microscopy to investigate changes in bacterial composition after a single session of FMRP instrumentation. They took samples at baseline, after 3, 7, 14, 21, 28, 35, 42, 49, 56, 70, and 90 days. After 3 days, a flora similar to periodontal health was found. It took motile cells 1 week, coccoid cells 21 days and spirochetes 42 days to return to baseline levels. In contrast, in the present study, the mean counts of *T. denticola* never reached

Table 3. Clinical findings of the sampling sites, reductions of PPD and BOP and gain in RAL from baseline to 3 and 6 months (mean ± SD)

	B–3 months	Mean difference (FMRP-QRP) confidence interval	B–6 months	Mean difference (FMRP-QRP) confidence interval
PPD (mm)				
QRP	2.3 ± 0.57***	1.00 ± 0.44#	1.9 ± 1.02***	0.45 ± 0.77
FMRP	1.3 ± 1.25**	[0.82, 1.92]	1.5 ± 2.22	[− 1.17, 2.07]
RAL (mm)				
QRP	1.8 ± 1.01***	0.95 ± 0.49	1.2 ± 1.14**	0.50 ± 0.66
FMRP	0.8 ± 1.16	[− 0.07, 1.97]	0.7 ± 1.75	[− 0.89, 1.89]
BOP (%)				
QRP	70 ± 26***	35 ± 17	50 ± 33***	10 ± 19
FMRP	35 ± 47*	[− 1, 71]	40 ± 52*	[− 31, 51]

$n_{(QRP)} = 10$ patients, $n_{(FMRP)} = 10$ patients.
*$p < 0.05$,
**$p < 0.01$,
***$p < 0.001$; p-values represent longitudinal changes from baseline within QRP and FMRP groups.
#p < 0.05; p-values represent differences between QRP and FMRP
(Comparisons of values after 3 and 6 months with baseline values are based on one-sample t-tests comparing the mean changes with zero). QRP, quadrant wise scaling and root planing (control group); FMRP, full-mouth scaling and root planing (test group); BOP, bleeding on probing; PPD, probing pocket depth; RAL, relative attachment level.

baseline levels. Sbordone et al. (1990) using darkfield microscopy and cultivation, analysed subgingival plaque samples with a curette at baseline and 7, 21, and 60 days after a single session of scaling and root planing and found an almost complete rebound after 60 days for *F. nucleatum, P. gingivalis*, and *P. intermedia*. This was not observed in the present study, where the mean counts of *F. nucleatum, P. gingivalis*, and *P. intermedia* never reached baseline levels during the 24 weeks. In the studies by Sbordone et al. (1990) and Mousquès et al. (1980), no attempt was made to alter the existing oral hygiene habits of the subjects. In contrast, as shown in a more recent study, using phase-contrast microscopy and cultivation for analysis of bacteria, under strict plaque control, no significant increase of total CFU and selected target bacteria was found on comparing values immediately post-instrumentation and after 1 and 2 weeks (Rhemrev et al. 2006). These findings were confirmed by the present study, where repeated plaque control was carried out.

Several studies compared the microbiological effects of full-mouth disinfection (Quirynen et al. 1999, De Soete et al. 2001) and/or full-mouth root planing (Quirynen et al. 2000, Apatzidou et al. 2004, Koshy et al. 2005) with quadrantwise root planing. These reports showed differing results; the studies by Quirynen et al. (1999, 2000) and De Soete et al. (2001) demonstrated advantages for the full-mouth approach (with or without use of chlorhexidine) *versus* quadrantwise treatment. The present study could not confirm these findings; in contrast, in concordance with the results of Apatzidou et al. (2004) and Koshy et al. (2005), treatment modalities showed a similar impact on target bacteria. The comparison between the present study and previous reports is complicated due to differences with respect to sampling time points, sampling methods, identification methods, and detection limits. Quirynen et al. (1999, 2000), Koshy et al. (2005) and the present study sampled plaque with paper points, and Apatzidou et al. (2004) with curettes. Also, sampling time points differed between the studies; Quirynen et al. (1999, 2000) and Koshy et al. (2005) took plaque samples no earlier than 1 month after baseline samplings. Apatzidou et al. (2004) took the first plaque samples apart from baseline 6 weeks after the last instrumentation. In the present study, sampling was performed eight times, five times alone within the first month, because during this time the greatest risk for re-infection of treated sites from untreated periodontal lesions had to be assumed. To the best of our knowledge, no other studies have evaluated the effects of QRP *versus* FMRP on these early microbiological events.

Other important factors for the differing results may be the state of oral hygiene, the amount of calculus, the degree of inflammation, and the extent of periodontal destruction in the different studies. Patients in the studies of Quirynen et al. (1999, 2000) and De Soete et al. (2001) were instructed in oral hygiene after the first session of scaling and root planing; later, no further sessions of oral hygiene were performed. Patients in the studies of Apatzidou et al. (2004) were instructed to achieve optimal oral hygiene, and patients treated in the study of Koshy et al. (2005) and the present study were instructed at least at one visit to perform adequate oral hygiene before treatment started; oral hygiene was repeatedly reinforced throughout the study period. Therefore, the risk for cross-contamination in the control group seems to be somewhat higher in the Leuven than in the other studies (Quirynen et al. 2006). This assumption is further supported by earlier studies that have demonstrated that in the absence of supragingival plaque control, re-colonization of the periodontal pocket takes place within 2–8 weeks after treatment (Magnusson et al. 1984, Sbordone et al. 1990). Also, patients in the study of van Winkelhoff et al. (1987) were not instructed in oral hygiene during an 8-week study using a single course of scaling and root planing. Although a statistically significant reduction of percentages of *P. gingivalis*, spirochetes and motile rods after 2 weeks was achieved; at 8 weeks the percentages of spirochetes and motile rods were elevated significantly. *P. intermedia* increased continuously during their study. Interestingly, comparing their data of *P. gingivalis* with the results of the present study, the same trend after 2 weeks can be recorded. After 8 weeks, an ongoing reduction was found, although more pronounced in the FMRP group. Regarding *P. intermedia* in the present study, an increase was noticed after 2 weeks in both groups, which corresponds well with the data of van Winkelhoff et al. (1987). But, after 8 weeks, the percentages of *P. intermedia* decreased in contrast to the findings of van Winkelhoff et al. (1987), indicating a possible influence of the good oral hygiene performed by the patients in the present study.

The outcome of microbiological investigations depends on the identification method used for microbiological analysis (Loomer 2004). Various methods for microbiological analysis of subgingival plaque have been used comparing full-mouth *versus* partial-mouth treatment. Quirynen et al. (1999, 2000)

used for microbiological investigation differential phase contrast microscopy and analysis with bacterial cultivation, which may have some limitations in identification of subgingival periodontal microorganisms (Eick & Pfister 2002, Jervøe-Storm et al. 2005, Boutaga et al. 2006). De Soete et al. (2001) used checkerboard DNA–DNA hybridization for the identification of 30 subgingival bacteria from 31 patients treated in former studies by Quirynen et al. (1999) and Mongardini et al. (1999). Apatzidou et al. (2004) and Koshy et al. (2005) used PCR for bacterial identification. The present study used a novel real-time PCR method which enables quantification of subgingival bacteria (Jervøe-Storm et al. 2005). The threshold level was adjusted to 10^3 bacteria/plaque sample in an attempt to establish a clinically relevant threshold level for the various periodontal pathogens (Doungudomdacha et al. 2001, Darout et al. 2003). In fact, the real-time PCR-based test used in this study has an even lower detection limit, which might not be of clinical relevance.

The use of molecular biological methods with their high sensitivity and specificity should be taken into consideration for analysis of periodontal pathogens (Boutaga et al. 2006). Real-time PCR has been introduced for the identification of *A. actinomycetemcomitans* (Sakamoto et al. 2001, Morillo et al. 2003, Lau et al. 2004, Nonnenmacher et al. 2005), *P. gingivalis* (Lyons et al. 2000, Sakamoto et al. 2001, Boutaga et al. 2003, Morillo et al. 2003, Lau et al. 2004, Nonnenmacher et al. 2005), *P. intermedia* (Nonnenmacher et al. 2005), *T. denticola* (Sakamoto et al. 2001, Asai et al. 2002, Yoshida et al. 2004) and *T. forsythia* (Sakamoto et al. 2001, Lau et al. 2004).

The present study showed reductions of approximately one log TBC/plaque sample, which is in agreement with expected reductions after non-surgical treatments described in the literature. The possible detection of dead bacteria does not pose a fundamental problem. Moreover, the statistical analysis compared the microbiological changes following both treatments starting at first day. It is very unlikely that greater amounts of dead bacteria were still present in the pocket 24 h after treatment, due to the sulcus fluid flow rate.

In conclusion, the present study could not confirm that treated sites were at a higher risk for bacterial re-infection in the presence of yet untreated periodontal lesions as is the case in QRP when compared with FMRP within 24 h. The results of the present study showed similar microbiological outcomes following both treatment modalities.

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Clinical Relevance

Scientific rationale for the study: In an attempt to prevent early re-infection from untreated sites, alternative protocols for subgingival scaling and root planing have been proposed. Controversial results have been reported for the microbiological effects of full-mouth disinfection and full-mouth root planing *versus* the standard quadrantwise approach.

Principal findings: This study could not confirm any differences in re-colonization after scaling and root planing within 24 h compared with treatment over several sessions.

Practical implications: Both modalities showed comparable changes in the subgingival microbiological status.