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Immediate effect of instrumentation on the subgingival microflora in *deep inflamed pockets under strict plaque control*

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Abstract

Objective: To investigate (1) reduction in the number of microorganisms obtained directly after subgingival instrumentation, (2) rate of bacterial re-colonization during 2 weeks, under supragingival plaque-free conditions.

Materials and Methods: Effects of subgingival instrumentation were measured at one deep pocket in 22 patients (11 smokers and 11 non-smokers). Immediately after initial therapy, experimental sites, under strict plaque control, were instrumented subgingivally. Microbiological evaluation was performed at pre-instrumentation, immediate post-instrumentation and 1 and 2 weeks post-instrumentation.

Results: Mean total anaerobic clolony forming units (CFUs) dropped from 3.9×10^6 before to 0.09×10^6 immediately following instrumentation. Significant reductions were found for *Tannerella forsythia, Micromonas micros, Fusobacterium nucleatum* and spirochetes. Significant reductions were not observed for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Campylobacter rectus*. Except for spirochetes, no reduction in prevalence of specific periodontal bacteria was found immediately after instrumentation. During follow-up, mean total CFU tended to increase. Prevalence of periodontal bacteria further reduced. No effect of smoking was found.

Conclusion: Results indicate that subgingival mechanical cleaning in itself, has a limited effect, in actually removing bacteria. The subsequent reduction in prevalence of specific periodontal bacteria shows that it is apparently difficult for these species to survive in treated pockets.

Key words: periodontal therapy; periodontitis; re-colonization; smoking; subgingival microflora

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It is generally agreed that the success of periodontal therapy depends on the reduction of periodontal pathogens in the subgingival area. Studies have shown that subgingival debridement in periodontitis patients is followed by profound shifts in the composition of the subgingival microflora which is comparable with the microflora found in healthy sites (Listgarten & Helldén 1978, Slots et al. 1979, Haffajee et al. 1985, Lavanchy et al. 1987). However, these changes are transient, especially in residual deep sites (probing pocket depth (PPD) $\geqslant$ 6 mm) after periodontal therapy (Slots et al. 1979, Magnusson et al. 1984, van Winkelhoff et al. 1988). Re-colonization of the subgingival area by motile rods and spirochetes may occur within 2–8 weeks (Mousqués et al. 1980, Magnusson et al. 1984, Van Winkelhoff et al. 1988), which seems to be dependent on the level of oral hygiene of the patient, the efficacy of the subgingival debridement and the residual PPD (Waerhaug, 1978, Magnusson et al. 1984, Van Winkelhoff et al. 1988, Sbordone et al. 1990). A positive association has been demonstrated between low proportions of

periodontal pathogens and a reduction in pocket depth and gain in probing attachment after periodontal treatment (Slots et al. 1979, Van Winkelhoff et al. 1988, Pedrazzoli et al. 1991, Renvert et al. 1996, Haffajee et al. 1997). After periodontal therapy pockets are re-populated by a microflora that is dominated by *Actinomyces* species and *Streptococcus* species (Slots et al. 1979, Pedrazzoli et al. 1991, Haffajee et al. 1997). In addition, it was found that at subgingivally untreated sites, in treated patients, there is a trend towards a decrease for some periodontal pathogens (Pawlowski et al. 2005). In contrast, when subjects have high post-treatment plaque levels outgrowth by various periodontal pathogens can reach pre-treatment levels in 3 weeks (Sbordone et al. 1990, Pedrazzoli et al. 1991). Another factor that may influence the pace of subgingival bacterial re-colonization is smoking. Data from the literature are conflicting. Studies show little, if any, influence on the subgingival occurrence of bacteria most commonly associated with periodontal disease (Darby et al. 2000, Boström et al. 2001, Natto et al. 2005). Outcome of non-surgical therapy in smokers and non-smokers was equally effective with regard to reducing periodontal pathogens (Preber et al. 1995). However, results of other studies indicate that smoking does influence the prevalence of periodontal pathogens after periodontal therapy (Grossi et al. 1996, Haffajee et al. 1997, Eggert et al. 2001, Van Winkelhoff 2001, Van der Velden et al. 2003). In this perspective, it is hard to support expectations with regard to the influence of smoking on the short-term re-colonization of periodontal pockets immediately after therapy. To possibly trace and, when necessary, correct for a smoking effect stratification of the patient cohort for smoking may be considered obligatory.

So far, little information is available in the literature regarding the microbiological effects of scaling and root planing in the period immediately after therapy in the absence of supragingival plaque. Therefore, the aim of the present study was (1) to investigate the reduction in the number of microorganisms that is obtained directly after subgingival instrumentation, and (2) to study the rate of bacterial re-colonization during the subsequent 2 weeks under conditions free of supragingival plaque, in a cohort that was balanced for smoking habits.

Materials and Methods

Patient and pocket selection

For the present study, 22 untreated periodontitis patients (mean age 44.8 years, SD:11.8) were selected from those referred to the Department of Periodontology of Academic Centre for Dentistry Amsterdam (ACTA) for treatment of periodontal disease. Exclusion criteria to enter the study were, the use of antibiotics during the past 6 months, pregnancy and systemic diseases that could possibly influence the condition of the periodontal tissues and the subgingival microflora. Inclusion criteria were the presence of moderately deep pockets (PPD $\geqslant$ 5 mm) and generalized bleeding on probing. As smoking could influence the results, the study group was balanced for smoking and included 11 smokers and 11 non-smokers. Of the non-smokers, nine never smoked, whereas two subjects had stopped smoking more than 1 year before intake. Mean number of pack years of the smokers cohort was 17.6 (SD: 14.7), and the number of years of smoking ranged from 10 to 44 years. After screening, all participants were requested to provide written-informed consent.

For the present study, one experimental site per patient was selected that had to fulfill the following criteria: (1) the deepest inter-proximal site with PPD $\geqslant$ 5 mm and bleeding on probing located at a single-rooted tooth, (2) shallow buccal and lingual probing depths (PPD $\leqslant$ 4 mm) on the same tooth, and (3) absence of any angular bony defects exceeding 45° as visible on periapical radiographs.

Treatment

Phase I

In order to obtain supragingival plaque-free conditions at the experimental sites during the experiment, treatment was divided into two phases. Phase I started at intake and consisted of periodontal examination, oral hygiene instructions and supragingival instrumentation at all teeth. Subgingival instrumentation was carried out at all teeth except two, i.e. the selected tooth and the tooth adjacent to the experimental site of the selected tooth. Use of a powered-tooth brush (Oral-B, Braun®, Kronberg/Ts, Germany) was instructed and inter-dental brushes, synthetic gauze and dental floss or tape as appropriate. The patients' plaque control was monitored weekly. A maximum of six sessions of 1 h/week were scheduled for periodontal treatment depending on the severity and the extent of the disease. During phase I supra- and subgingival instrumentation was provided using ultrasonic and/or hand instruments.

Phase II

Phase II started 1 week after completion of phase I. In phase II, a single episode of subgingival instrumentation was performed at the selected tooth and the tooth adjacent to the experimental site of the selected tooth. This procedure was carried out, by one experienced clinician (I. V.), using only hand instruments. Microbiological assessments were performed at pre-instrumentation, immediate post-instrumentation and 1 and 2 weeks after instrumentation. Patients received oral hygiene instructions at every appointment. During these 2 weeks of observation the patients rinsed twice daily with chlorhexidine mouthrinse 0.2% (Corsodyl®, Zeist, the Netherlands) and were instructed to use an inter-dental brush moistened with the CHX fluid between the selected teeth in order to perform a maximal supragingival plaque control at these sites. Clinical measurements at the experimental sites were obtained at intake, pre-instrumentation and 1 and 2 weeks after instrumentation. The clinical measurements included:

1. supragingival plaque accumulation (Silness & Löe 1964).
2. bleeding index. Bleeding on probing: 0, no bleeding; 1, pin-prick bleeding; and 2, immediate and overt bleeding.
3. PPD (in mm) using a standard periodontal probe (Hu-Friedy®, Chicago, IL, USA).

All clinical and microbial assessments were carried out by one examiner (G. R.), who was not present during the subgingival instrumentation procedures.

Microbiological procedures

Sampling

Sites were subjected to careful removal of supragingival plaque deposits with a scaler. To avoid saliva contamination the selected area was isolated with cotton rolls and gently air dried. Before bacterial sampling a Hu-Friedy

periodontal probe was inserted in the approximal pocket, along the axis of the tooth until definite resistance was met. Two endodontic paper points (size 40#, Johnson & Johnson, Windsor, NJ, USA), were subsequently inserted for 15 s each, into the pocket along the probe, taking into account not to fold or to push it into another area. The paperpoints were both transferred into the same vial containing 1.8 ml of reduced transport fluid (RTF) (Syed & Loesche 1972). During the experiments the room temperature varied between 18 and 24°C (Petit et al. 1991). Time between sampling and the microbial analysis was kept to a minimum and never exceeded 1 h.

Microscopy

The sample was homogenized in a Vortex mixer for 30 s and then dispersed by aspirating the suspension five times through a tuberculin syringe (1 ml Terumo syringe with a 0.45 × 12 mm neolus needle). One drop of the suspension was placed in a Thoma bright line counting chamber (Hawksley, Lancing, UK) with squares covering 1/400 mm^2 (depth 0.02 mm) and coverslipped. Total number spirochetes per sample was assessed, as described by Listgarten & Helldén (1978). The samples were examined by means of a phase-contrast microscope equipped with a heat filter at a magnification of × 1200. Counting the spirochetes was performed in randomly selected squares until at least 40 grid squared were counted.

Culture

Samples were cultured for further microbiological analysis on the same day. Samples were vortexed for 30 s and 10-fold serially diluted in reduced transport fluid; 0.1 ml of each dilution was plated on 5% horse blood agar plates supplemented with haemin (5 mg/l) and menadione (1 mg/l) for determination of the total anaerobic bacterial counts and specific periodontal pathogens.

Samples were subsequently plated on trypticase soy serum–bacitracin–vancomycin plates (TSBV) for isolation and counting of *Actinobacillus actinomycetemcomitans* (Slots 1982). TSBV plates were incubated in air with 5% CO_2 at 37°C for 5 days; blood agar plates were incubated for 14 days at 37°C in 80% N_2, 10% CO_2 and 10% H_2 (Van Steenbergen et al. 1986). Presence and proportions of the putative periodontal pathogens *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *M. micros* and *Campylobacter rectus* were determined on the anaerobic blood agar plates (van Winkelhoff et al. 1985). Identification of the selected bacterial species was based on the Gram stain and cell and colony morphology, air tolerance, and production of catalase and on a number of biochemical reactions (Van Winkelhoff et al. 1986). *A. actinomycetemcomitans* was identified on the basis of its characteristic colony morphology (star-like inner structure), a positive catalase reaction with 3% hydrogen peroxide, and a set of specific enzymes. The total viable count (TVC) was estimated on the horse blood agar plates from the dilution giving 30–300 colonies, and was expressed as the number viable count per millilitre of transport medium.

Data analysis

A repeated measures analysis was used to analyse effects on clinical parameters in time with smoking as a covariate. Wilcoxon's-signed-ranks test were used to explore the effect of treatment on both clinical and microbiological data for differences over time. McNemar tests were applied in order to test the effect of treatment on the prevalence of the various bacteria over time. To explore possible differences in microbiological parameters between smokers and non-smokers non-parametric tests were used as appropriate. As no significant effect for smoking could be found for any of the parameters, the test statistics of McNemar and Wilcoxon's tests were pooled to weight the results for non-significant effects of smoking. p-values <0.05 were considered statistically significant.

Results

Clinical findings

The clinical parameters of the experimental sites at intake and during phase II are presented in Table 1. In phase I, during the observation period from intake to pre-instrumentation, the clinical parameters improved significantly. At pre-instrumentation, the plaque scores were almost zero. During phase II, a gradual further reduction in bleeding and PPD was observed throughout the 2 weeks of observation. In this period, almost no plaque was present. However at week 2, still 45% of the sites showed bleeding after probing. No statistically significant effect of smoking was found for any of the clinical parameters for the entire study period.

Microbiological findings

The results of the effect of instrumentation on the subgingival microflora during phase II are presented in Table 2. Immediately after instrumentation almost no effect on the prevalence of the specific periodontal bacteria was found except for the spirochetes, which showed a significant reduction in prevalence. At 2 weeks after instrumentation, however, a reduction in the prevalence of *P. gingivalis*, *P. intermedia*, *M. micros* and *F. nucleatum* was seen. The prevalence of *A. actinomycetemcomitans* ($N = 4$) and *C. rectus* ($N = 4$) remained unchanged while *T. forsythensis* was present in 16 patients before instrumentation and dropped to lower levels after instrumentation. However, statistical significance was achieved only at 1-week post-instrumentation for this

Table 1. Means (SD) of clinical parameters at the experimental sites ($N = 22$) during phases I and II of the study

	Phase I		Phase II	
	intake	pre-instrumentation	One-week post-instrumentation	Two-week post-instrumentation
Plaque index	1.82 ± 0.39	0.09 ± 0.29*	0.00 ± 0.00*	0.05 ± 0.21*
Bleeding index	1.91 ± 0.29	1.45 ± 0.51*	0.73 ± 0.70*†	0.64 ± 0.66*†§
PPD (mm)	5.91 ± 0.75	5.36 ± 0.79*	5.14 ± 0.89*†	4.91 ± 0.97*†§

*Significant difference from intake (Wilcoxon's $p<0.05$).
†Significant difference from pre-instrumentation (Wilcoxon's $p<0.05$).
§Significant differences from pre-instrumentation (repeated measures analysis, intake and smoking as covariates).

species ($N = 8$). Before and immediately after instrumentation nine subjects were found positive for *P. gingivalis*. This number was significantly reduced to four subjects at 2 weeks after instrumentation. Similarly *P. intermedia, M. micros* and *F. nucleatum* showed no significant reduction immediately after instrumentation but further significant reductions were observed at 1 or 2 weeks. Spirochetes were found in nine patients before instrumentation. Immediately after instrumentation this number dropped to one and remained unchanged for the following 2 weeks. Smoking had no statistically significant effect on any of the bacteria examined.

The results for the numbers of bacteria during phase II are presented in Table 3. Mean total anaerobic counts as determined by culture at pre-instrumentation was 3.784×10^6/ml CFUs. Immediately after instrumentation total CFUs were significantly reduced to a mean of 0.092×10^6/ml. After instrumentation throughout the 2 weeks of observation no more significant changes in the total number of CFUs could be determined. During phase II no statistically significant reduction in CFUs in patients positive for *A. actinomycetemcomitans*, *P. gingivalis, P. intermedia* and *F. nucleatum* was found. In patients positive for *T. forsythia*, *M. micros* and *F. nucleatum* mean CFUs were significantly reduced immediately after instrumentation. For *T. forsythia* and *F. nucleatum* mean CFUs remained suppressed throughout the 2 weeks of observation. The mean number of spirochetes at pre-instrumentation was 12.1×10^6/ml, and immediately after instrumentation this was significantly reduced to 0.1×10^6/ml. Throughout the 2-week observation period mean counts remained significantly suppressed. Also during phase II no statistically significant effect of smoking was found.

Table 2. Prevalence of specific periodontal bacteria for the study group ($N = 22$) during phase II of the study

	pre-instrumentation.	Immediate post-instrumentation	One-week post-instrumentation	Two-week post-instrumentation
Actinobacillus actinomycetemcomitans	4	4	3	4
Porphyromonas gingivalis	9	9	6	5*†
Prevotella intermedia	5	3	2*†	2*†
Tannerella forsythia	16	12	8*	10
Micromonas micros	21	18	14*	15*
Fusobacterium nucleatum	19	17	14	12*
Campylobacter rectus	4	5	4	5
spirochetes	9	1*	1*	2*

*Significantly different from pre-instrumentation ($p < 0.05$, McNemar test).
†Significantly different from immediate post-instrumentation ($p < 0.05$, McNemar test).

Discussion

Previous studies have shown that the re-colonization of the subgingival area by microorganisms may occur within 2–8 weeks after treatment (Mousques et al. 1980, Magnusson et al. 1984, Van Winkelhoff et al. 1988, Wade et al. 1992). It became also clear that the presence of supragingival plaque plays a pivotal role in this process (Magnusson et al. 1984, Van Winkelhoff et al. 1988, Sbordone et al. 1990, Pedrazzoli et al. 1991). In order to study the rate of subgingival bacterial re-colonization properly, the influence of supragingival plaque should be eliminated. As plaque development is both dependent of periodontal inflammation (Goh et al. 1986, Quirynen et al. 1991, Ramberg et al. 1994, 1995, Rowshani et al. 2004, Dahan et al. 2004) and numbers of bacteria in the saliva (Dahan et al. 2004) the design of the present study included pre-experimental periodontal treatment of the whole dentition except for the experimental sites. In addition, in phase II of the study the patients were asked to rinse two times a day with chlorhexidine and to apply an inter-dental brush moistened with chlorhexidine at the experimental site. The extremely low-plaque scores of the present study make it unlikely that the subgingival re-colonization in this study was influenced by supragingival plaque.

Results of microbiological studies are highly dependent on the sampling procedure. It has been shown that the composition of the microflora may change with the distance from the gingival margin (Listgarten 1976, Slots 1979, Magnusson et al. 1984). A major problem in the present study was, that because of the treatment, the periodontal tissues would tighten around the teeth (Beardmore 1963). As a consequence, it would become more difficult to intro-

Table 3. Mean total CFU/ml ($10^6 \pm$ SD), mean numbers of specific cultural periodontal bacteria (CFU/ml, $10^6 \pm$ SD) and numbers of spirochetes ($10^6 \pm$ SD) of positive patients during phase II of the study

	Pre-instrumentation.	Immediate post-instrumentation	One-week post-instrumentation	Two-week post-instrumentation
Total CFU/ml	3.78 ± 8.98	0.09 ± 0.18*	0.15 ± 0.27*	0.23 ± 0.42*
Actinobacillus actinomycetemcomitans	0.18 ± 0.33	0.01 ± 0.01	0.05 ± 0.09	0.02 ± 0.04
Porphyromonas gingivalis	1.01 ± 1.55	0.01 ± 0.03	0.04 ± 0.10	0.04 ± 0.1
Prevotella intermedia	0.01 ± 0.04	0.001 ± 0.003	0.0003 ± 0.0005	0.0002 ± 0.0003
Tannerella forsythia	1.55 ± 5.93	0.004 ± 0.006*	0.006 ± 0.02*	0.0007 ± 0.002*
Micromonas micros	0.33 ± 0.84	0.01 ± 0.02*	0.03 ± 0.06	0.04 ± 0.1
Fusobacterium nucleatum	0.12 ± 0.28	0.003 ± 0.006*	0.01 ± 0.03*	0.002 ± 0.005*†
Campylobacter rectus	0.03 ± 0.05	0.002 ± 0.005	0.01 ± 0.02	0.01 ± 0.04
spirochetes	12.1 ± 28.3	0.10 ± 0.3*	0.1 ± 0.4*	0.50 ± 1.7*

*Significantly different from pre-instrumentation ($p < 0.05$, Wilcoxon's test).
†Significantly different from 1 week after instrumentation ($p < 0.05$, Wilcoxon's test).

duce a paper point to the bottom of a pocket. In fact, in a pilot study extremely high reductions in the number of subgingival bacteria were found after instrumentation because of the inability to introduce the paper point deeply into the pocket after therapy. Therefore, the sampling technique was modified by using a periodontal probe, as a guide for the paper points. These were inserted subsequently along the tip of the probe, thereby not folding or pushing the paper point away from the bottom of the pocket. In this way, an attempt was made to standardize the sampling procedure. In all samples, the examiner was capable of inserting the paper points up to the end of the probe tip.

The results showed an improvement of clinical parameters of the experimental sites between intake and pre-instrumentation, although these sites had not been subgingivally cleaned and only received stringent supragingival plaque control measures. In particular, PPD showed a reduction of about 0.5 mm. Data of Christou et al. (1998) demonstrated a comparable change in PPD in patients with moderate-to-severe periodontitis after the use of inter-dental brushes not accompanied by any further instrumentation. Furthermore, improvement of yet untreated sites in the course of full-mouth periodontal treatment has been described in several studies (Cercek et al. 1983, Dahlén et al. 1992, Hellström et al. 1996). Pawlowski et al. (2005) found that untreated sites in treated patients tended to improve clinically over a 24-week period but that the levels of *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* remained unchanged while levels of *P. intermedia* and *Treponema denticola* tended to decrease but increments failed to reach the level of significance. Unfortunately that study did not report the probing depth of the experimental sites. In an other study, supragingival professional cleaning three times a week over a 6-month period in subjects with 4–5-mm-deep pockets resulted in a decrease of *P. gingivalis* and spirochetes (McNabb et al. 1992). Furthermore, supervised self-performed supragingival plaque control exercised over extended periods of time (2–4 years) at sites $\leqslant 6$ mm also showed a decrease in specific periodontal bacteria (Dahlén et al. 1992, Sato et al. 1993). Such an effect was not found in deeper sites $\geqslant 6$ mm (Beltrami et al. (1987). As the experimental sites of the present study included pockets $\geqslant 5$ mm, the subgingival microbiological profile may have partly improved during phase I of the study, although the time lapse between the completion of phase I and the start of phase II was only 1 week and phase I itself included only 4–6 weeks. Unfortunately, no microbiological examination was carried out at intake and therefore no comments can be made regarding this aspect of the present study. A further improvement of the clinical condition of the experimental sites was seen after phase II. However, the improvement of the clinical parameters obtained between the start of phase I and the end of phase II was still less as compared with the results in the literature with regard to the effect of initial periodontal therapy (Cercek et al. 1983, Badersten et al. 1984, Magnusson et al. 1984,). In these studies the observation time was much longer, so it is likely that a 2-week period of observation is too short to obtain a result of a magnitude comparable with the effects as described in these studies.

No statistically significant effects of smoking were found for any of the clinical parameters. The literature suggests a more pronounced effect of smoking, as most studies have shown that non-smokers show a greater pocket depth reduction compared with smokers (Ah et al. 1994, Boström et al. 1998, Machtei et al. 1998, Renvert et al. 1998). However, changes in clinical parameters over the 2 weeks of observation in the present study are most likely not of a magnitude that possible influences of smoking can already be discerned at the level of statistical significance, even in this well-balanced but limited cohort.

The dental literature contains few studies that deal with the ''true'' early re-colonization following instrumentation. Mousquès et al. (1980), using darkfield microscopy, found major changes in the proportional bacterial composition 3 days after instrumentation. These changes lasted up to 42 days after the treatment. Wade et al. (1992) evaluated the effect of instrumentation on the basis of culturing. They started the subgingival assessments 1 week following treatment. Their results showed a two to three fold reduction in CFUs at the 1- and 2- week assessments. In the present study, a larger effect was observed, i.e. 25- and 16-fold reduction, respectively, at the 1- and 2- week assessments. Part of these differences in observations may be the result of a difference in baseline CFUs. In the study Wade et al. (1992) this was 1.6×10^6 whereas, in the present study this was 3.8×10^6. As far as we are aware of, to date no information has been presented in the literature regarding the reduction in numbers of microorganisms immediately following subgingival instrumentation. The results of the present study show that immediately after instrumentation a 42-fold reduction in total CFU was obtained. For the specific periodontal bacteria this varied between no significant reduction in CFUs to an almost 400-fold decrease (*T. forsythia*). However, as immediately after instrumentation only the spirochetes exhibited a reduction in prevalence, and the total CFU still amounted to 10^5/ml these results indicate that subgingival mechanical cleaning in itself, has a limited effect in removing bacteria. During the 2-week follow-up, the mean total CFU showed a trend to increase whereas a further reduction in the prevalence of specific periodontal bacteria could be observed. The latter phenomenon could hypothetically be explained by disruption of the subgingival biofilm by the instrumentation resulting in non-adhering bacteria that can be more easily phagocytosed by PMNs in the pocket. It has been shown that among smokers more patients remain positive for specific periodontal bacteria after periodontal therapy compared with the non-smokers (Grossi et al. 1996, Van der Velden et al. 2003). One explanation, among others, may be that the PMN function is impaired in smokers (Kenney et al. 1977, Pabst et al. 1995). In the present study no differences were found between smokers and non-smokers with regard to the bacteriological results. One reason for this finding may be related to the relatively short evaluation period of 2 weeks. In this period from directly after subgingival instrumentation until 2 weeks, a decrease in the prevalence of periodontal bacteria was found. It could be hypothesized that in non-smokers a further reduction may occur during the following period whereas this is not the case in smokers.

In conclusion, the results of the present study indicate that subgingival mechanical cleaning in itself has a limited effect in actually removing bacteria. The subsequent reduction of the specific periodontal bacteria shows that it is apparently difficult for these bacteria to

survive in a cleaned pocket. This may be because of changed ecological conditions and also the host immune response in general.

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Clinical Relevance

Scientific rationale: It has been well documented that thorough subgingival scaling and root planing will result in a reduction of periodontal bacteria. However in the presence of supragingival plaque, rapid subgingival recolonization will occur within a few weeks. The present study was conducted to test the microbiological effects of scaling and root planing in the period immediately after therapy in the absence of supragingival plaque.

Principal findings: Directly after instrumentation almost no effect on the prevalence of periodontal pathogens was found. However, 2 weeks after instrumentation a reduction of the prevalence was found for most of the periodontal pathogens. This may suggest that the host response is an important factor with regard to the subgingival microbial flora after treatment.