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# Microbiological findings after periodontal therapy using curettes, Er:YAG laser, sonic, and ultrasonic scalers

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***Abstract***

**Aim:** To evaluate and compare the microbiological effects of hand instruments, Er:YAG-laser, sonic, and ultrasonic scalers in patients with chronic periodontitis. Patient perception of each treatment was documented.

**Material and Methods:** From 72 patients, bacterial samples were collected from the deepest pocket in each quadrant (total: 288 sites). A polymerase chain reaction kit estimated the amount of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Tannerella forsythensis* (*Tf*), and *Treponema denticola* (*Td*) at baseline as well as 3 and 6 months after therapy. One quadrant in each patient was randomly assigned to curettes (H-group), Er:YAG laser (L-group), sonic device (S-group), or ultrasonic device (U-group).

**Results:** Three months post-operatively, the amounts of *Pg*, *Pi*, *Tf*, and *Td* were significantly reduced in all groups. Laser and sonic instrumentation failed to reduce *Aa*. Six months after therapy, significant differences were still detected for *Pg* (L- and U-group), for *Pi* and *Tf* (S-group), and for *Td* (L-, S- and U-group). Patients rated ultrasonic treatment as more preferable than hand and laser instrumentation.

**Conclusion:** The various treatment methods resulted in a comparable reduction of the evaluated periodontal pathogens, and bacterial increase was only partially different 6 months post-operatively. Ultrasonic instrumentation caused less discomfort.

Key words: chronic periodontitis; Er:YAG laser; non-surgical mechanical therapy; periodontal pathogens; polymerase chain reaction; randomized clinical trial; supportive maintenance care

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Numerous clinical and microbiological studies have confirmed that non-surgical mechanical treatment, consisting of plaque control and mechanical debridement, is effective in reducing the bacterial load, thus resulting in clinical improvement of the periodontal disease. Chronic periodontitis is caused by mixed infections with the subgingival microbiota being organized as a biofilm and characterized by a continual flux. Therefore, no single pathogen can be considered responsible for the aetiology and pathogenesis of periodontitis. Instead, in particular the subgingival species *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Tannerella forsythensis* (*Tf*), and *Treponema denticola* (*Td*) have been suggested as putative periodontal pathogens contributing to the disease (Haffajee & Socransky 1994).

Hence, adequate removal of pathogenic bacteria from the supra- and subgingival environment by non-surgical mechanical periodontal therapy is required for optimal healing of the diseased periodontal tissues (Cugini et al. 2000). Thorough scaling and root planing (SRP) of patients' teeth showing moderate-to-severe periodontitis with hand instruments have consistently reported marked changes in the subgingival microflora and clinical indices following non-surgical instrumentation (Haffajee et al. 1997). Along with hand

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instruments, sonic and ultrasonic devices have been applied for subgingival mechanical debridement. Irrespective of the used instrument type, bacterial and calculus deposits can be removed by a scraping or hammering motion of the working tip towards the root surface, and many studies have shown a comparable treatment outcome with both hand and powered instruments (Drisko et al. 2000, Oda et al. 2004).

In order to improve effectiveness and efficacy of the removal of the subgingival biofilm, Er:YAG laser therapy has recently been recommended as an alternative to conventional scaling procedures (Ishikawa et al. 2004). The Er:YAG laser irradiation has been reported to exhibit high bactericidal properties (Folwaczny et al. 2002b). Furthermore, this laser has been assumed not only to eliminate bacteria but also to inactivate bacterial toxins diffused in the root cementum without producing a smear layer; this would undoubtedly contribute to improve periodontal health (Ishikawa et al. 2004).

Consequently, hand instruments, sonic and ultrasonic devices as well as the Er:YAG laser have been considered to be the main treatment approaches of non-surgical periodontal therapy (Ishikawa & Baehni 2004). Although numerous in vitro and in vivo studies examined the microbiological effects of these methods, and, to some extent, compared their bactericidal potential, no information with regard to their comparative effect on pathogenic bacteria in chronic periodontitis is available from the literature. Furthermore, there is a lack of information about the patients' perception of each treatment approach.

Thus, the aim of the present randomized-controlled single-masked clinical trial was to evaluate the microbiological effects of hand, laser, sonic, and ultrasonic instrumentation on reduction below the detection thresholds of *Aa*, *Pg*, *Pi*, *Tf*, and *Td* at the treated sites 3 and 6 months after therapy. Furthermore, this study aimed to document the patients' perception of each treatment modality with regard to pain, unpleasantness, and inconvenience experienced during the therapy.

## Material and Methods

### Patient selection

Owing to the lack of clinical investigations comparing primarily the decreasing effects of the four treatment modalities, a calculation of the sample size for this study was not possible. Thus, the estimation of the sample size was based on the clinical parameter ''clinical attachment level'' (CAL); this part of the study has already been published (for data, refer to Nonhoff et al. 2006). Based on 0.8 power to detect a difference of 0.25 mm (considered clinically significant) between the investigated groups ($p = 0.0083$; two-sided; Bonferroni's correction), and with an assumed loss to follow-up of 20%, 72 systemically healthy, volunteer patients who sought dental and periodontal treatment at the Department of Operative Dentistry and Periodontology, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, Germany, were included.

All recruited patients (42 females, 30 males), aged 28–76 years old (mean 53.8 ± 10.6 years), exhibited moderate-to-advanced chronic periodontitis, based on clinical and radiographic findings. Selection criteria included at least one pocket depth of 4 mm or more with bleeding on probing (BOP) and bone loss of at least one-third of the root length in each quadrant, along with very good oral hygiene scores (GI ⩽ 1 and PI ⩽ 1). Criteria for exclusion from the study were: periodontal treatment within the last 12 months; systemic diseases (e.g., cardiovascular disease, diabetes mellitus, rheumatoid arthritis, infection with HIV), which could influence the outcome of the therapy; history of systemic antibiotic therapy within the last 6 months or during the course of the study; pregnant or breast feeding women; residency outside the city of Berlin, insufficient address for follow-ups, or unwillingness to return for follow-ups; and diagnosis of another form of periodontitis.

The study was in accordance with the Helsinki Declaration of 1964 (as revised and amended in its sixth version in 2002). Approval of the study protocol by the Ethical Review Committee (vote number 214–28) at the Charité – Universitätsmedizin Berlin, Germany, was obtained, and (after adequately explaining the clinical trial and its aims, methods, anticipated benefits, potential risks of the study, and the discomfort it may entail) all participants signed appropriate informed consent forms.

### Study design

The study was performed according to a specific quadrant design. After screening of 223 patients, 151 subjects were excluded (Fig. 1), and a pre-trial oral hygiene phase (including up to three visits of toothbrushing instructions, along with teaching the use of interproximal cleaning aids such as floss and interdental brushes, depending on individual of the patients' needs) was completed. Subsequently, pocket probing depths (PPDs) were measured at six aspects of each tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual) using a manual periodontal probe (CP 15 UNC; Hu-Friedy, Chicago, IL, USA). In each quadrant, only one site with the deepest PPD (not shallower than 4 mm and without any endodontic or furcation involvement) was included. A total of 288 sites of single- and multi-rooted teeth were microbiologically assessed at each visit. Each quadrant in every patient was randomly assigned using a randomization list (SPSSWIN® 10.0; SPSS Inc., Chicago, IL, USA) generated at the Department of Biometry and Clinical Epidemiology (Charité – Universitätsmedizin Berlin) into each one of the following four debridement modalities: hand instruments (H-group), Er:YAG laser (L-group), sonic scalers (S-group), and ultrasonic scalers (U-group). All patients were treated by the same experienced operator (F. D.) within 24 h. Subgingival plaque samples were taken from each site at baseline as well as 3 and 6 months post-operatively.

To assure the blinded character of the study, the patients were supervised by one examiner (J. N.) and one operator (F. D.). Each patient had an examination file as well as a treatment file. The examination file provided information about the clinical measurements at each examination period and was accessible only to the examiner, who was kept unaware of the intervention. The treatment file included data about the randomization of the treatment modalities and documented the process or completion of the therapy. Only the operator had access to that file. Thus, operator and examiner were blinded to each other. The patients were not told which method of subgingival debridement was used in the respective quadrants. As it can be assumed that some patients could guess the treatment modality, the present study was solely single blinded.

### Oral hygiene programme

For 3–5 weeks before start of the study all patients were enrolled in a hygiene

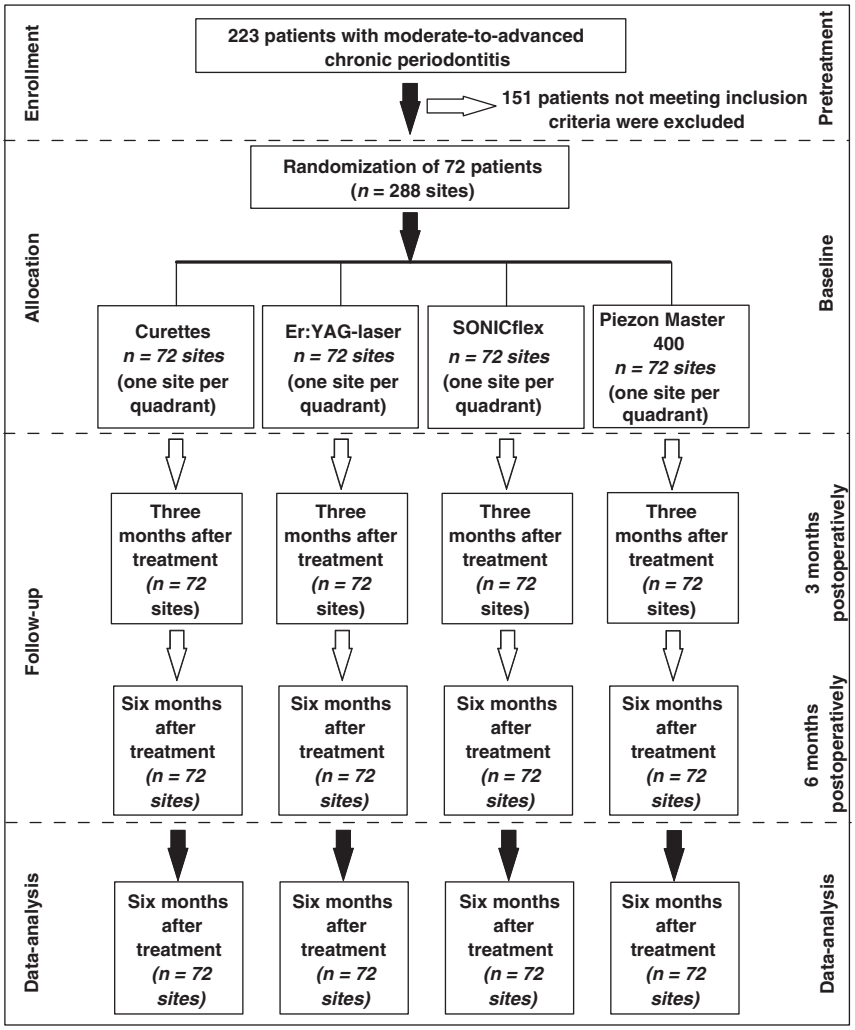


*Fig. 1.* Flowchart giving the phases of the present clinical trial.

programme, and received oral hygiene instructions on three to five appointments as well as supragingival scaling and polishing according to individual needs. To compensate for potential individual variations in plaque control among the subjects and to reduce pathogens present on other oral surfaces, all patients were instructed to rinse twice a day with a 0.1% chlorhexidine solution. Additionally, to minimize potential bacterial seeding sources during the course of the study, all carious lesions, defective dental restorations, and pulpal pathology were eliminated pre-operatively.

#### Clinical measurements

After the pre-treatment phase, the following clinical parameters were measured by one calibrated periodontist (J. N.) in order to investigate the oral hygiene of the patients and to detect the deepest pocket in each quadrant: plaque index (PI) (Silness & Löe 1964), gingival index (GI) (Löe & Silness 1963), and PPD (measured in mm from the crest of the gingival margin to the bottom of the pocket). The PPD measurements were made at six aspects per tooth using a CP 15 UNC (Hu-Friedy). The examiner was not involved in providing any treatment during the study.

#### Examiner calibration

Five patients with two pairs of contralateral (single- and multi-rooted) teeth with PPD $>4$ mm on at least one aspect of each tooth were recruited for the calibration. The examiner performed measurements over two visits within 48 h at the same side of each tooth. The calibration was successfully completed if measurements at baseline and at 48 h were similar to the millimetre at a >90% level. Statistical analysis for the quality of the calibration procedures was not conducted.

#### Microbiological assessment

A total of 288 subgingival plaque samples were collected before clinical measurements at baseline and at 3 and 6 months' follow-ups, respectively. Identification of the target microorganisms (*Aa*, *Pg*, *Pi*, *Tf*, and *Td*) was accomplished utilizing a polymerase chain reaction (PCR)/DNA probe test (micro-IDent test; Hain Lifescience, Nehren, Germany) (Eick & Pfister 2002). One microbiological sample was taken at each of the four patients' teeth with the deepest probing depths. After ensuring that no supragingival plaque deposits were present, the sites were isolated with sterile cotton rolls, cleaned supragingivally with sterile cotton pellets, and gently air dried in an apico-coronal direction. Subgingival plaque samples were taken with one sterile paper point that was inserted into the sites to be examined. Care was taken to access the most apical part of the pocket with the paper point and to prevent cross-contamination. The paper point remained in the pocket for 20 s, and was subsequently transferred to a sterile transport vial.

Analysis was performed at the microbiology laboratory of Hain Diagnostika (Nehren, Germany), according to standardized procedures. For the bacterial determination, DNA probes were used with a multiplex PCR, followed by hybridization against a quantitative probe as described previously (Eick & Pfister 2002). Clinical examinations as well as bacteriological sampling were carried out by one masked and calibrated examiner being unaware of what treatment the quadrants of the patients had. Microbiological assessment and analysis were performed blinded.

According to the manufacturer's recommendations, cut-offs for *A. actinomycetemcomitans* were set to $10^3$ and for *P. gingivalis*, *P. intermedia*, *T. forsythensis*, and *T. denticola* to $10^4$ genome equivalents per probe. According to other studies (Papapanou et al. 1997, Tomasi et al. 2006, Xajigeorgiou et al. 2006), the estimated bacterial amounts were transformed into a scale

of scores (ranging from 0 to 4) as follows:

- Score 0: $<10^3$ for *Aa*, $<10^4$ for *Pg*, *Pi*, *Tf* and *Td*
- Score 1: $=10^3$ for *Aa*, $10^4$ for *Pg*, *Pi*, *Tf*, and *Td*
- Score 2: $\leqslant 10^4$ for *Aa*, $\leqslant 10^5$ for *Pg*, *Pi*, *Tf*, and *Td*
- Score 3: $\leqslant 10^5$ for *Aa*, $\leqslant 10^6$ for *Pg*, *Pi*, *Tf*, and *Td*
- Score 4: $>10^5$ for *Aa*, $>10^6$ for *Pg*, *Pi*, *Tf*, and *Td*.

#### Therapeutic procedures

On the basis of the quadrant design of this study, each quadrant in every patient was randomly allocated to one of the four non-surgical treatment groups. Thus, each treatment regimen was assigned to 72 sites.

Hand instrumentation was performed using both standard and Mini-Five™ Gracey curettes (Hu Friedy) as deemed necessary by the clinician. All instruments were re-ground after each working cycle with the PerioStar 3000 unit (Hawe Neos, Bioggio, Switzerland).

An Er:YAG laser device (wave length 2.94 $\mu$m) (KEY 3; KaVo, Biberach, Germany) in combination with a calculus detection system (fluorescence induced by 655 nm; InGaAsP diode laser radiation) was selected for laser treatment using an energy level of 160 mJ/pulse and a repetition rate of 10 Hz with water irrigation according to the manufacturer's instructions (Schwarz et al. 2001, 2003, Sculean et al. 2004, Tomasi et al. 2006). The treatment was performed from coronal to apical with fibre tips of $0.5 \times 1.65$ and $0.5 \times 1.1$ mm. The inclination of the fibre tip to the tooth surface was 15–20° (Folwaczny et al. 2001).

Sonic instrumentation was conducted using the SONICflex® system (LUX 2003 L; KaVo) operating with an increasing amplitude (120, 150 and 240 $\mu$m), and a frequency of 6000 Hz with constant water irrigation according to the manufacturer's instructions. The subgingival debridement was performed using three different tips (Nr. 60, 61, 62), which were inserted into the periodontal pocket parallel to the root surface.

The piezoelectric device Piezon Master 400 (EMS, Nyon, Switzerland) was used for ultrasonic instrumentation. The debridement was performed using three different ceramic tips (PS, PL1, PL2), operating at a frequency of 28.000 Hz with constant water cooling.

All treatments were performed under local anaesthesia by the same experienced operator (F. D.) within 24 h. Laser instrumentation was terminated when the detection system indicated the absence of deposits on the root surface; this was the case, if the relative intensity of fluorescence as induced with diode laser radiation was below the threshold value of 5 (Folwaczny et al. 2002a, 2004). The subgingival debridement with curettes, sonic and ultrasonic scalers was performed until the operator subjectively felt that the root surfaces were adequately debrided and planed. The mean time needed in the laser group was 6.5 min. for single-rooted teeth and 11 min. for multi-rooted teeth. For the hand instrumentation, the averages were 9.7 min. for single-rooted and 15.3 min. for multi-rooted teeth. The amount of time required for the sonic and ultrasonic instrumentation was 7.3 and 8.2 min. for single-rooted teeth, respectively, and 13.4 and 14.6 min. for multi-rooted teeth.

#### Maintenance care

All patients received dental prophylaxis at 2-week intervals for the first three post-operative months and once a month thereafter. This consisted of oral hygiene instructions in self-performed plaque-control measures and supragingival scaling and polishing.

#### Patients perception

Information about experienced pain, unpleasantness, or inconvenience upon completion of each treatment approach was obtained by the use of a questionnaire. The patients' perception was presented on a visual analogue scale graded from 0 to 10, where the left end point (0) indicated severe (''very painful'', ''very unpleasant'' and ''very inconvenient'') and the right end point (10) represented no discomfort (''not painful'', ''very pleasant'', and ''not inconvenient''). The patients were asked to place a mark in the appropriate position on the line. The distance from the no-point was then measured with a millimetre ruler. Immediately after each mode of treatment, the patients completed a new standardized questionnaire; thus, patients were not influenced by the previous results (Braun et al. 2003, Hoffman et al. 2005). To investigate whether the patients' acceptance of the respective treatment method remained the same compared with the one directly after completion of the treatment, patients were asked to assess treatment once again 1 month post-operatively.

#### Statistical analysis

Because this study utilized a split-mouth design, the unit of analysis was the site to be examined. Data were entered into an Excel sheet database (MS Office Excel 2003; Microsoft, Redmond, WA, USA) and were proofed for entry errors. The statistical evaluations were conducted using SAS® Version 9.1 (SAS Institute Inc., Cary, NC, USA). The mean, standard deviation, and standard error for all variables were determined and data were tested for normal distribution. Changes of the bacteria amounts between different points of time (baseline, 3 and 6 months) as well as between the four different treatment groups were determined using the GENMODE procedure. Correlations of data arising from measurements of several units at the same patient were estimated using the generalized estimation equations (GEE) model. Microbial data were expressed as mean scaled scores from 0 to 4 as described above. Statistical evaluations of the subjective patient perception of pain, unpleasantness or inconvenience after each treatment approach were computed using non-parametric tests (Friedman's test; Wilcoxon's test). Because each pair of the four treatment groups was tested, the local level of statistical significance was adjusted to $\alpha = 0.0083$ (Bonferroni's correction, factor 6). A post hoc power calculation analysis was conducted with respect to the detectable differences between the treatment methods at a power level of 0.8 (nQuery Advisor® Version 3.0; Statistical Solutions Ltd., Broadway, Saugas, MA, USA).

## Results

#### Descriptive statistics of the sites to be examined

Participants were recruited from July 2003 to September 2004, and attended clinic visits at the time of randomization (baseline) as well as after 3- and 6-month intervals. Of the 72 patients recruited and included to this trial (Fig. 1), all completed the active periodontal therapy, and could be followed up to the end of the study; none of the sites to be treated were lost during the study. From

*Table 1.* Distribution of the moderate (4–6 mm) as well as of the deep (⩾7 mm) pockets in the four treatment groups at baseline

| Initial PPD | Curettes | Laser | Sonic | Ultrasonic |
|---|---|---|---|---|
| 4–6 mm | 40 (55.6%) | 47 (65.3%) | 47 (65.3%) | 46 (36.1%) |
| ⩾7 mm | 32 (44.4%) | 25 (34.7%) | 25 (34.7%) | 26 (63.9%) |
| Total | 72 (100.0%) | 72 (100.0%) | 72 (100.0%) | 72 (100.0%) |

Numbers and percentage of the pockets are given.
PPD, pocket probing depths.

*Table 2.* Pre-operative distribution of the mean values ( ± SE) of the scaled bacterial amounts (as having been detected both in moderate (4–6 mm) and in deep (⩾7 mm) pockets) in the four treatment groups

| Initial PPD 4–6 mm | Curettes | Laser | Sonic | Ultrasonic |
|---|---|---|---|---|
| *Aa* | 1.13 ( ± 0.19) | 0.83 ( ± 0.18) | 1.00 ( ± 0.22) | 1.24 ( ± 0.24) |
| *Pg* | 1.80 ( ± 0.20) | 1.81 ( ± 0.18) | 1.66 ( ± 0.20) | 1.91 ( ± 0.18) |
| *Pi* | 0.60 ( ± 0.17) | 0.74 ( ± 0.17) | 0.96 ( ± 0.20) | 0.85 ( ± 0.18) |
| *Tf* | 1.38 ( ± 0.19) | 1.47 ( ± 0.17) | 1.45 ( ± 0.16) | 1.76 ( ± 0.18) |
| *Td* | 1.03 ( ± 0.17) | 1.09 ( ± 0.14) | 1.28 ( ± 0.15) | 1.16 ( ± 0.15) |

| Initial PPD ⩾7 mm | Curettes | Laser | Sonic | Ultrasonic |
|---|---|---|---|---|
| *Aa* | 0.97 ( ± 0.27) | 1.16 ( ± 0.30) | 0.48 ( ± 0.24) | 0.92 ( ± 0.28) |
| *Pg* | 2.38 ( ± 0.20) | 2.52 ( ± 0.19) | 2.40 ( ± 0.23) | 2.62 ( ± 0.18) |
| *Pi* | 1.47 ( ± 0.28) | 1.16 ( ± 0.26) | 1.60 ( ± 0.28) | 1.27 ( ± 0.28) |
| *Tf* | 1.97 ( ± 0.18) | 2.36 ( ± 0.19) | 2.28 ( ± 0.17) | 1.92 ( ± 0.22) |
| *Td* | 1.59 ( ± 0.19) | 2.04 ( ± 0.18) | 1.96 ( ± 0.17) | 1.58 ( ± 0.19) |

PPD, pocket probing depths; *Aa*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola.*

*Table 3.* Number and percentage of sites harbouring the target species. (detection threshold for *Aa* $\geqslant 10^3$ cells/probe and for *Pg, Pi, Tf, Td* $\geqslant 10^4$ cells/probe)

| Species | Baseline | 3 Months | 6 Months |
|---|---|---|---|
| *Aa* | 91 (31.6%) | 75 (26.0%) | 94 (32.6%) |
| *Pg* | 223 (77.4%) | 167 (57.9%) | 203 (70.5%) |
| *Pi* | 126 (43.8%) | 76 (26.4%) | 104 (36.1%) |
| *Tf* | 221 (76.7%) | 142 (49.3%) | 196 (68.1%) |
| *Td* | 209 (72.6%) | 131 (45.5%) | 176 (61.1%) |

PPD, pocket probing depths; *Aa*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola.*

a total of 288 periodontal pockets, 72 received hand instrumentation (H-group), 72 laser therapy (L-group), 72 sonic scaling (S-group), and 72 were treated using ultrasonic instrumentation (U-group). Minimum probing depth in each group was 4 mm, and maximum values were 11 mm in the H-group, 12 mm in the L- and S-group, and 10 mm in the U-group. One hundred and eighty pockets (62.5%) had a probing depth of 4–6 mm, while 108 (37.5%) pockets were deeper than 7 mm.

To evaluate the distribution of the moderate (4–6 mm) as well as the deep (⩾7 mm) pockets among the four treatment groups before start of therapy, the initial PPDs were categorized according to the treatment received (see Table 1). Forty pockets with PPD 4–6 mm were treated using curettes, while for each 47 pockets the laser or the sonic instrumentation was used; 46 pockets were treated using the ultrasonic device. With the deep pockets (⩾7 mm), 32 were instrumented with curettes, 25 with laser, 25 with sonic, and 26 with ultrasonic.

Because the GEE analysis of the data at baseline did not consider the initial PDDs, this procedure did not reveal any statistically significant differences with respect to the distribution of the pre-operative bacterial amounts among the treatment groups. Table 2 presents the bacterial levels at baseline in all treatment groups in correlation with the initial PPDs. According to the results of the GEE procedure, there were no statistically significant differences among the four treatment approaches in terms of the distribution of the amount of each species.

#### Microbiologic effects of treatment

The numbers and percentage of the 288 sites harbouring each of the five target species at each examination period are given in Table 3. Each one of the five evaluated species was reduced 3 months after therapy, and increased its prevalence after 6 months. *Aa* exhibited the lowest prevalence at all examination periods. This species was detected in 31.6% of the sites at baseline, decreased after 3 months (26.0%), and increased again 6 months after therapy (32.6%). *Pg* was the most prevalent species at each examination (77.4% pre-operatively, 57.9% after 3 months, and 70.5% after 6 months). *Pi* was found in 43.8% of the sites at baseline, and could be detected in 26.4% and 36.1% of the sites 3 and 6 months after therapy, respectively. 76.7% of the sites harboured *Tf* pre-operatively, and this pathogen was observed with 49.3% and 68.1% of the sites at 3 and 6 months, respectively. At baseline, *Td* showed a greater prevalence (72.6%) than 3 (45.5%) and 6 months (61.1%) after therapy.

Overall, 90.3% of the sites were infected by one or more species at baseline; 3 months after therapy the incidence of the infected sites decreased to 70.8% and increased again slightly to 75.4% 6 months post-operatively (Table 4). Three and four species were harboured by the majority of the infected sites at baseline (29.9% and 35.4%, respectively). Three months after therapy the sites infected by three or four species decreased to 19.4% and 20.8%, respectively, while the sites infected by one or two species increased from 5.2% and 11.1% at baseline to 13.2% and 12.2%, respectively. At baseline, 8.7% of the sites were infected by five species (5.2% after 3 months of therapy). At 6 months post-operatively, the sites infected by one, two, three, four, or five species achieved almost the baseline values again.

#### Effects of each treatment approach on individual species

Table 5 shows the mean bacterial counts of each species as well as of the overall

bacterial numbers of the investigated species at baseline, 3 and 6 months post-operatively. Tables 6 and 7 give the differences of the mean number of the assessed bacteria amounts (scaled scores 0–4) between baseline and each post-operative examination period. At 3 months post-operatively, the level of *Aa* was significantly reduced in the H- and U-group ($p = 0.001$ and $p < 0.0001$, respectively). The reduction of the species was not significant after laser and sonic instrumentation ($p = 0.0154$ and 0.0688, respectively). All four treatment approaches significantly reduced the mean amount of *Pg* ($p < 0.0001$ for all treatment groups), *Pi* ($p = 0.0015$ for H-group, $p = 0.0059$ for L-group, $p < 0.0001$ for S-group, and $p = 0.0019$ for U-group), *Tf* ($p < 0.0001$ for all treatment groups), and *Td* ($p = 0.0002$ for H-group and $p < 0.0001$ for L-, S- and U-group) as well as the mean count of all five bacteria to be examined ($p < 0.0001$ for all treatment groups).

Six months after therapy, the bacteria amounts differently increased in each treatment group and for each species. The mean level of *Aa* at this examination period did not show any significant differences after hand, laser, sonic, or ultrasonic instrumentation, if compared with the baseline values ($p = 0.1038$, $p = 0.5028$, $p = 0.7216$, and $p = 0.0811$, respectively). The mean counts of *Pg* in the L and U group differed significantly from those at baseline ($p < 0.0001$). Compared with the baseline values, the amounts of *Pi* and *Tf* remained significantly reduced only in the S-group ($p = 0.0011$ and $p = 0.0002$, respectively). The mean counts of *Td* and the mean amounts of all five species showed a statistically significant difference in groups L ($p = 0.0031$ and $p = 0.0003$, respectively), S ($p = 0.0019$ and $p < 0.0001$, respectively), and U ($p = 0.0049$ and $p < 0.0001$, respectively) at this point of time when compared with baseline.

*Table 4*. Number (*N*) and percentage of sites infected by 1, 2, 3, 4, or 5 target species

| *N* Species | Baseline | 3 Months | 6 Months |
|---|---|---|---|
| 1 | 15 (5.2%) | 38 (13.2%) | 37 (12.8%) |
| 2 | 32 (11.1%) | 35 (12.2%) | 44 (15.3%) |
| 3 | 86 (29.9%) | 56 (19.4%) | 67 (23.3%) |
| 4 | 102 (35.4%) | 60 (20.8%) | 83 (28.8%) |
| 5 | 25 (8.7%) | 15 (5.2%) | 23 (8.0%) |
| Total | 260 (90.3%) | 204 (70.8%) | 254 (75.4%) |

#### Comparison of the microbial effect of each treatment modality

Analyses of the mean numbers of each species as well as of the mean numbers of all five bacteria at each examination period indicated that no significant differences among the four treatment groups were present in most cases; solely with regard to the amount of *Td*, the sonic treatment revealed a significantly higher reduction if compared with hand instrumentation 3 months after therapy ($p < 0.008$).

*Table 5*. Mean values of the scaled amounts ( ± SE) of each species as well as of all five species together at baseline as well as at 3 and 6 months after therapy

| Treatment groups | *Aa* | *Pg* | *Pi* | *Tf* | *Td* | All |
|---|---|---|---|---|---|---|
| Baseline | | | | | | |
| H | 1.14 ( ± 0.20) | 2.06 ( ± 0.15) | 0.99 ( ± 0.16) | 1.64 ( ± 0.14) | 1.28 ( ± 0.13) | 7.10 ( ± 0.49) |
| L | 0.81 ( ± 0.16) | 2.06 ( ± 0.14) | 0.89 ( ± 0.15) | 1.78 ( ± 0.14) | 1.42 ( ± 0.13) | 6.94 ( ± 0.43) |
| S | 0.82 ( ± 0.17) | 1.92 ( ± 0.16) | 1.18 ( ± 0.16) | 1.74 ( ± 0.13) | 1.51 ( ± 0.12) | 7.17 ( ± 0.46) |
| U | 1.13 ( ± 0.18) | 2.17 ( ± 0.14) | 1.00 ( ± 0.16) | 1.82 ( ± 0.14) | 1.56 ( ± 0.12) | 7.67 ( ± 0.43) |
| Three months after therapy | | | | | | |
| H | 0.72 ( ± 0.15) | 1.29 ( ± 0.14) | 0.60 ( ± 0.12) | 0.92 ( ± 0.12) | 0.82 ( ± 0.11) | 4.35 ( ± 0.46) |
| L | 0.57 ( ± 0.13) | 1.40 ( ± 0.13) | 0.50 ( ± 0.10) | 0.99 ( ± 0.12) | 0.69 ( ± 0.11) | 4.15 ( ± 0.42) |
| S | 0.61 ( ± 0.14) | 1.06 ( ± 0.13) | 0.36 ( ± 0.09) | 0.90 ( ± 0.13) | 0.64 ( ± 0.10) | 3.57 ( ± 0.39) |
| U | 0.65 ( ± 0.15) | 1.11 ( ± 0.14) | 0.56 ( ± 0.12) | 0.93 ( ± 0.13) | 0.82 ( ± 0.11) | 4.07 ( ± 0.45) |
| Six months after therapy | | | | | | |
| H | 0.92 ( ± 0.17) | 1.78 ( ± 0.14) | 0.83 ( ± 0.15) | 1.38 ( ± 0.13) | 1.13 ( ± 0.11) | 6.03 ( ± 0.43) |
| L | 0.89 ( ± 0.16) | 1.63 ( ± 0.14) | 0.61 ( ± 0.13) | 1.46 ( ± 0.13) | 1.03 ( ± 0.11) | 5.61 ( ± 0.43) |
| S | 0.86 ( ± 0.16) | 1.54 ( ± 0.13) | 0.68 ( ± 0.11) | 1.29 ( ± 0.13) | 1.08 ( ± 0.12) | 5.46 ( ± 0.41) |
| U | 0.92 ( ± 0.17) | 1.44 ( ± 0.14) | 0.75 ( ± 0.13) | 1.44 ( ± 0.13) | 1.18 ( ± 0.12) | 5.74 ( ± 0.44) |

PPD, pocket probing depths; *Aa*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola*.

*Table 6*. Differences of mean bacteria amounts (scaled scores 0–4) ( ± SE) between baseline and 3 months post-operatively in each treatment group

| Treatment groups | *Aa* | *Pg* | *Pi* | *Tf* | *Td* | All |
|---|---|---|---|---|---|---|
| H | **0.42 ( ± 0.13)** | **0.76 ( ± 0.14)** | **0.39 ( ± 0.12)** | **0.72 ( ± 0.14)** | **0.46 ( ± 0.12)** | **2.75 ( ± 0.44)** |
| L | 0.24 ( ± 0.10) | **0.65 ( ± 0.11)** | **0.39 ( ± 0.14)** | **0.72 ( ± 0.12)** | **0.72 ( ± 0.11)** | **2.79 ( ± 0.32)** |
| S | 0.21 ( ± 0.11) | **0.86 ( ± 0.15)** | **0.82 ( ± 0.16)** | **0.83 ( ± 0.14)** | **0.88 ( ± 0.12)** | **3.60 ( ± 0.45)** |
| U | **0.47 ( ± 0.12)** | **1.06 ( ± 0.14)** | **0.44 ( ± 0.14)** | **0.89 ( ± 0.15)** | **0.74 ( ± 0.12)** | **3.60 ( ± 0.42)** |

Bold fonts represent significant differences.

PPD, pocket probing depths; *Aa*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola*.

*Table 7.* Differences of mean bacteria amounts (scaled scores 0–4) ( ± SE) between baseline and 6 months post-operatively in each treatment group

| Treatment groups | *Aa* | *Pg* | *Pi* | *Tf* | *Td* | All |
|---|---|---|---|---|---|---|
| H | 0.22 ( ± 0.14) | 0.28 ( ± 0.14) | 0.15 ( ± 0.12) | 0.26 ( ± 0.14) | 0.15 ( ± 0.13) | 1.07 ( ± 0.41) |
| L | − 0.08 ( ± 0.12) | **0.43 ( ± 0.10)** | 0.28 ( ± 0.14) | 0.32 ( ± 0.14) | **0.39 ( ± 0.13)** | **1.33 ( ± 0.36)** |
| S | − 0.04 ( ± 0.12) | 0.38 ( ± 0.16) | **0.50 ( ± 0.20)** | **0.44 ( ± 0.12)** | **0.43 ( ± 0.14)** | **1.71 ( ± 0.44)** |
| U | 0.21 ( ± 0.12) | **0.72 ( ± 0.13)** | 0.25 ( ± 0.13) | 0.38 ( ± 0.14) | **0.38 ( ± 0.13)** | **1.93 ( ± 0.39)** |

Bold fonts represent significant differences.
PPD, pocket probing depths; *Aa*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola*.

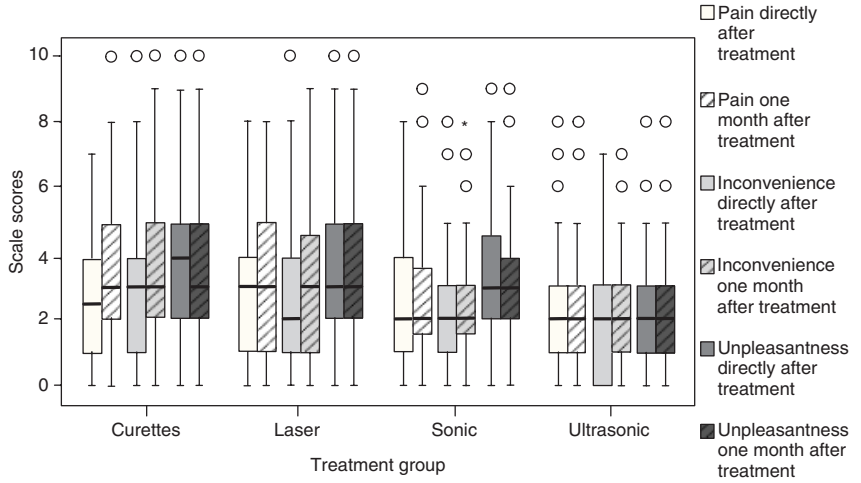


*Fig. 2.* Box-and-whiskers plots representing the patient perception for each debridement method directly after completion of treatment as well as after 1 month. Boxes illustrate median, as well as upper and lower quartiles. The remaining values are given by whiskers, except for outliers (circles = values between 1.5 and three box-lenghts from the boundaries of the box) and extreme cases (*defined as values more than three box-lenghts from the boundaries of the box).

#### Post hoc power analysis

The post hoc power analysis showed that, after 3 and 6 months, the minimal detectable difference between the four treatment modalities for each species as well as for all five bacteria was too low to meet the statistical requirement of a power level of 0.8 (0.46, 0.45, 0.56, 0.55, 0.47, 1.64 for *Aa*, *Pg*, *Pi*, *Tf*, *Td*, and for all species, respectively).

#### Patient perception

Analysis of the data obtained from the questionnaire on pain, unpleasantness, or inconvenience (experienced by the patients during the various treatment methods) revealed that patients perceived sonic debridement significantly more pleasant than the hand or laser instrumentation ($p < 0.001$ and $p = 0.002$, respectively) (Fig. 2). This result was confirmed by the second survey 1 month later. In addition, here the patients described the treatment with curettes as significantly more painful than with the ultrasonic tips ($p = 0.001$); with regard to inconvenience and unpleasantness, hand instrumentation was ranked significantly worse if compared with the ultrasonic and sonic devices ($p = 0.007$ and $p < 0.001$, respectively) (Fig. 2).

## Discussion

#### Study design

The use of split-mouth designs in periodontal studies has been controversially discussed in the past. Indeed, the main purpose of any split-mouth design is to remove all components related to differences between subjects from the treatment comparisons. The advantage of within-patient comparisons (in contrast to between-patient comparisons) is that the error variance (noise) of the experiment can be reduced, thereby obtaining a higher statistical power (Hujoel & Loesche 1990), along with smaller numbers of patients required for the trial (Hujoel & DeRouen 1992). However, due to the fact that the oral cavity can be considered as an open system (with microbiological interactions that may occur), an interacting effect of each treatment can be assumed. It has already been reported that at untreated sites in an otherwise treated mouth, a 30% improvement in clinical parameters (including decreased counts in some bacterial species) can occur (Pawlowski et al. 2005). Notwithstanding, an (indeed conceivable) interacting influence in case of multiple mechanical debridement methods has not been assessed up to now. Thus, more studies are needed to investigate the carry-across effects of different treatment modalities; in case the sums of the carry-across effects of all treatments are the same, the estimated treatment difference should be unbiased.

By means of these controversies and under consideration of the smaller number of patients required, the present clinical trial utilized a split-mouth design. This quadrant design facilitated the evaluation of separate units for each studied treatment approach. To minimize limitations of the split-mouth design, the GEE methodology was utilized. The results of the GEE procedure did not reveal any significant differences regarding the distribution of each species among the four treatment modalities. Before start of therapy, a homogenous distribution of the bacteria was observed in all treatment groups, if the data were analysed with regard to both groups of initial PPDs (Table 2).

Moreover, the distribution of PPDs of 4–6 mm was comparable among the experimental units (Table 1). This finding was in agreement with the results of former investigations (Hujoel & Loesche 1990). Accordingly, the deeper pockets ($\geqslant 7$ mm) in this study were similarly distributed among the laser, sonic, and ultrasonic groups, and only

the curettes group included a slightly increased number of deeper pockets if compared with the other groups (Table 1). Thus, the disease severity exhibited a reasonably comparable distribution amongst the quadrants (Imrey 1986). In addition, this study utilized a blinded randomization process to overcome the concerns of possible disparities of the experimental units and to avoid any bias (Chalmers et al. 1983).

With respect to the time points of evaluation, this study accomplished the post-operative examinations 3 and 6 months after therapy, in order to deliver results with clinical relevance; the rationale for the 3-month intervals is based on the suggestion of previously published studies reporting that this frequency of supportive maintenance care is necessary to reduce subgingival proportions of pathogens (Renvert & Persson 2004). Moreover, the post-operative microbiological analysis was performed with regard to completion of the healing processes.

#### Microbiological effects of treatment modalities 3 months after therapy

With regard to the antimicrobial effects of the four different treatment modalities, a separate analysis of moderate (initial PPD 4–6 mm) and deep (PPD $\geqslant$7 mm) pockets revealed a comparable outcome for each treatment approach on the respective species. Moreover, this was according to the results observed without concerning the initial PPDs. Therefore, the results presented here do not discriminate against the microbiological effects of the treatment groups according to the initial PPDs.

Specific microbial associations have been observed not only between different bacteria but also amongst bacterial complexes; for example, it is extremely uncommon to find red complex species (*Pg*, *Tf*, *Td*) in the absence of members of the orange complex (i.e. *Pi*) (Socransky et al. 1998). This is obviously the reason why in this study the majority of the sites were pre-operatively infected by four species. It has been shown that bacterial re-growth and re-colonization of the pockets occur, with bacterial counts being restored almost to pre-treatment values 3–7 days after treatment (Harper & Robinson 1987), but with a clearly altered composition of the subgingival microflora. Early colonizers of the dental plaque usually occupy the pockets much faster, thus inhibiting the establishment of more pathogenic species (Cugini et al. 2000). This could explain why in this study more sites were infected by one or two species after therapy, whereas the majority of sites harboured three or four species at baseline.

In this study, hand instrumentation resulted in a significant reduction of the amount of each species as well as of all five species 3 months after therapy. Accordingly, Er:YAG laser radiation significantly reduced the numbers of *Pg*, *Pi*, *Tf*, and *Td* as well as the total count of the investigated bacteria after 3 months; however, laser treatment obviously failed to completely eliminate the bacteria from the periodontal pockets. Moreover, a significant reduction of *Aa* could not be detected. Hence, a differing decrease of bacteria numbers was obtained in this study for the various species, and this finding was in accordance with a recent investigation (Eberhard et al. 2003). Different sensitivities to laser radiation has been attributed to variations in morphology and water content of the bacteria, and level of pigmentation of the cell walls (Folwaczny et al. 2002b).

With the sonic instrumentation, the subgingival quantity of *Pg*, *Pi*, *Tf*, and *Td* as well as the total amount of the five species could be significantly affected at 3 and 6 months. Again, the amount of *Aa* did not show any significant changes if compared with baseline. Although the effects of acoustic microstreaming produced by the sonic scalers should enable the clinician to remove the subgingival plaque effectively (Khambay & Walmsley 1999), the limited effects with regard to *Aa* were probably due to the ability of the species to invade the soft tissues (Zambon 1985).

Moreover, it could be speculated that the DNA analysis used in this investigation had detected genetic material of *Aa* of both living and dead cells (with the latter not having been removed from the periodontal pockets). Hence, with regard to the post-operative number of the evaluated amount of *Aa*, no significant reduction of the numbers of *Aa* could have been revealed. However, subgingival sampling was taken 3 months after active periodontal treatment was completed, and bacterial counts for *Aa* were indeed reduced in the H and U groups. Thus, the low pre-operative numbers of sites infected by *Aa* in the laser and sonic group present a much more reliable explanation for this discrepancy.

Ultrasonic instrumentation has been shown to be as effective as the use of manual scalers in removal of subgingival plaque and calculus, without any difference regarding the clinical outcome (Drisko et al. 2000, Oda et al. 2004). Moreover, ultrasonic scalers show a better accessibility to furcations and grooves (Oda et al. 2004), and possess a cavitational activity, which has been considered effective for removal of plaque and endotoxins as the theoretical adjunct to the mechanical action of the instrument (Walmsley et al. 1988); however, this could not be verified clinically (Petersilka & Flemmig 1999). In accordance with other investigations (Braun et al. 2006), ultrasonic debridement revealed a significant reduction of *Pg*, *Pi*, *Tf*, and *Td* as well as of the amount of all five bacteria in the present study; additionally, the detectable values of the more-resistant *Aa* were significantly decreased after 3 months. For the red complex species, significant differences compared with baseline values could be shown even for the 6-month evaluation.

More studies are needed to interpret and explain the significantly higher reduction of *Td* in the sonic group if compared with the curettes 3 months after instrumentation in this study. This was in contrast to earlier investigations not demonstrating any superiority of the sonic scalers to the hand instruments (Laurell 1990), when using culture techniques and dark-field microscopy as analytical methods. Moreover, comparisons between hand and ultrasonic instrumentation (Oosterwaal et al. 1987, Copulos et al. 1993) as well as between ultrasonic debridement and sonic instrumentation (Baehni et al. 1992) failed to show any differences with regard to bacterial reduction. Correspondingly, comparable results were observed for comparisons between Er:YAG laser and hand instruments (Schwarz et al. 2001) as well as between Er:YAG laser and power-driven scalers (Tomasi et al. 2006). An explanation for this finding could be the above-mentioned limitation of the utilized split-mouth design (i.e. possible interacting effects of the four treatment methods).

Non-surgical mechanical periodontal therapy was effective in controlling moderate-to-advanced chronic periodontitis after 3 months (Nonhoff et al. 2006). According to the already published short-term clinical data of this study, gingival recession (GR), PPD,

and CAL showed a statistical significant difference at 3 months post-operatively if compared with baseline ($p < 0.0001$; GEE) in all treatment groups. With regard to bacteria reduction below the detection threshold, previous clinical trials did not show any differences between laser and hand instruments (Eberhard et al. 2003), or between oscillating scalers and hand instruments (Braun et al. 2006). Accordingly, this study failed to detect any differences among treatments over the observation period; all treatment methods led to a reduction of the bacterial amounts. However, it should be stressed that the findings of the post hoc power calculation analysis indicated that the present investigation did not have adequate power to detect a clinically relevant difference between the four approaches if a real difference existed. Therefore, this study has indeed some pilot character, and clinical trials with an adequate sample size should follow to investigate as primary outcome measures the microbiological effects of the different treatment modalities of non-surgical periodontal therapy.

#### Microbiological effects of treatment modalities 6 months after therapy

Six months after therapy, the bacteria amounts differently increased in each treatment group and for each species. Starting from a striking low prevalence of 31.6% (thus indicating the chronic form of periodontitis), the numbers of *Aa* returned to the previous levels in all treatment groups; accordingly, this could be observed with *Pg* after laser and ultrasonic instrumentation, with *Pi* and *Tf* in the curettes, laser and ultrasonic group, and with *Td* and the overall of all studied species after hand instrumentation.

It should be emphasized that all patients in this study had completed active treatment for chronic periodontitis, and maintained low plaque and inflammation scores throughout the duration of the study (Nonhoff et al. 2006). Thus, the patients' own infection control seemed to be satisfactory; additionally, a supportive treatment regimen was implemented on a fortnight's basis, and both strategies have been considered as crucially important to successfully maintaining periodontal stability (Kornman et al. 1994).

However, the bacterial reductions achieved in this study 3 months after therapy could not be maintained. It is well accepted that perio-pathogens cannot be completely eradicated by conventional SRP, and the deeper the pocket, the more incomplete should be the removal of subgingival plaque and calculus, at least with hand instruments (Izumi et al. 1999). Nonetheless, proportions of up to 10% of the total flora and counts smaller than $10^5$ have been associated with periodontal health (Ximenez-Fyvie et al. 2000).

With regard to increase of the bacterial amounts, the periodontal pocket seems to be the primary source of bacterial translocation. Owing to their ability to invade gingival tissues, the perio-pathogens can form ''reservoirs'', thus increasing the survival of the microorganisms in the periodontal tissues and promoting their transmission (Arakawa et al. 2000). It could be assumed that the maintenance of low plaque and inflammation scores decelerated the subgingival increase of bacteria having survived the periodontal therapy. Therefore, the bactericidal effects of the periodontal treatment were still detectable 3 months post-operatively (but not at 6 months after treatment). In addition, bacteria may reside in root surface irregularities and dentinal tubules, as well as in other sites of the oral cavity, in particular in extra-dental areas such as the dorsal surface of the tongue, buccal mucosa, palate, and tonsils (Quirynen et al. 2001). Moreover, the role of an extra-oral source cannot be totally ruled out (Greenstein & Lamster 1997). These considerations should be taken into account when interpreting antimicrobial effects. In total, no statistical differences were found between the four treatment modalities.

#### Patient perception

With regard to patient comfort, there are only limited data comparing different types of instrumentation. In this study, all patients were treated using (repeated) local anaesthesia. Therefore, it seems clear that evaluation of true pain perception was not reliable. However, usual circumstances during treatment (e.g., type of instrument, stress on jaws or neighbouring teeth, opened mouth or the sidewise turned head) are parameters possibly influencing the patients' perception with respect to inconvenience (and pain). In addition, smells, noises, vibrations as well as unusual taste after treatment might have affected the characterization of each treatment method as pleasant or unpleasant.

Nonetheless, statistical analysis of the patient questionnaire at both examination periods (directly after finishing the therapy and 1 month later) revealed a significant difference in patients' perception of treatment in this study: ultrasonic treatment was considered more pleasant as well as less painful and inconvenient than debridement with hand instruments. This was in accordance with recent studies (Braun et al. 2003, Kocher et al. 2005). Owing to their modified design, modern ultrasonic scalers show excellent access to deep pockets and furcation areas. Although newly developed hand instruments facilitate SRP therapy, the manual subgingival debridement is strongly influenced by the shape of the instrument (and that of the pocket or the root surface) as well as by the skills of the operator (Breininger et al. 1987, Kepic et al. 1990). Moreover, the patients of this study favoured ultrasonic treatment over the significantly more unpleasant laser instrumentation, which was obviously due to unpleasant, but temporary, smells. Despite the copious water irrigation used with the laser instrumentation, these unpleasant smells could not be reduced to a desirable, non-detectable minimum in some cases.

In total, patient satisfaction is generally regarded as an indicator of quality of health care; analysis of the subjective patient evaluation of each method showed that sonic and ultrasonic instrumentation obtained a higher patient acceptance than debridement with the curettes or the laser.

## Conclusions

Within the limitations of this study, it may be concluded that the four non-surgical treatment modalities – curettes, Er:YAG laser, sonic, and ultrasonic scalers – resulted in a significant reduction of the amounts and prevalence of the five pathogenic species *Aa*, *Pg*, *Pi*, *Tf*, and *Td* 3 months after therapy. Six months after active periodontal therapy, the amount of bacteria increased again to a varying extent in each treatment group and for each species. Clinical trials should follow to determine the microbiological effects of each treatment modality, in particular with regard to the advanced Er:YAG laser device, which did not prove superior in this

study. According to the patients' perceptions, the ultrasonic instrumentation was judged more favourable than subgingival debridement using hand instruments or laser treatment.

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**Clinical Relevance**

*Scientific rationale for the study*: Clinical trials comparing directly the microbiological effects of curettes, Er:YAG laser, sonic, and ultrasonic scalers on chronic periodontitis as well as the patient perception of each treatment are lacking.

*Principal findings*: Three months post-operatively, the amounts of each species as well as of all five pathogens together were significantly reduced in each group; 6 months after therapy bacteria amounts increased again, but without any significant differences between the four treatment modalities. Patients rated ultrasonic instrumentation as more preferable.

*Practical implications*: The studied treatment approaches have similar effects on perio-pathogenic bacteria. Concerning patient's acceptance, ultrasonic scaling is favoured.