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# Periodontal debridement as a therapeutic approach for severe chronic periodontitis: a clinical, microbiological and immunological study

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***Abstract***

**Aim:** To clinically, microbiologically and immunologically characterize periodontal debridement as a therapeutic approach for severe chronic periodontitis.

**Material and Methods:** Twenty-five patients presenting at least eight teeth with a probing pocket depth (PPD) of $\geqslant 5$ mm and bleeding on probing (BOP) were selected and randomly assigned to quadrant-wise scaling and root planing or one session of full-mouth periodontal debridement. The following clinical outcomes were assessed: plaque index, BOP, position of gingival margin, relative attachment level (RAL) and PPD. Real-time PCR was used for quantitative analysis of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia*. The enzyme-linked immunosorbent assay permitted the detection of IL-1$\beta$, prostaglandin $E_2$, INF-$\gamma$ and IL-10 in gingival crevicular fluid (GCF). All the parameters were evaluated at baseline, and at 3 and 6 months after treatment.

**Results:** Both the groups had similar means of PPD reduction and attachment gain over time. Besides a significant reduction in the bacterial level after treatment in both groups, microbiological analysis failed to demonstrate significant differences between them. Finally, no difference was observed between groups with respect to the levels of inflammatory mediators in GCF.

**Conclusion:** Periodontal debridement resulted in a similar clinical, microbiological and immunological outcome when compared with standard scaling and root planing and therefore may be a viable approach to deal with severe chronic periodontitis.

Key words: chronic periodontitis; debridement; randomized clinical trial; root planing; scaling; ultrasonic

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The removal of microbial biofilm, calculus, ''contaminated'' root cementum and dentin has been the basis for periodontal therapy (O'Leary 1986). Scaling and root planing is the most common therapy for periodontitis and, in association with adequate supragingival biofilm control, has well-documented success (Badersten et al. 1984). However, this treatment modality is time consuming, demands a high level of operator skill and is unpleasant for patients (Kinane 2005). Furthermore, the traditional scaling and root planing performed in a quadrant or sextant-wise manner with a gap of 1 or 2 weeks between appointments also facilitates the recolonization of treated areas by pathogens residing in untreated pockets and other extra-dental sites (Quirynen & Bollen 1995).

An alternative strategy to treat chronic periodontitis has been proposed as an attempt to decrease bacterial recolonization in treated sites (Quirynen &

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Bollen 1995). Basically, this approach consists of scaling and root planing within 24 h, associated with an extensive anti-microbial regime with chlorhexidine. This protocol was named one-stage full-mouth disinfection. Later, it was concluded that the conduction of treatment within 24 h was the effective aspect of the therapy, rather than oral chlorhexidine disinfection (Quirynen et al. 2000). Quirynen et al. (2006) observed that the completion of scaling and root planing within a short time frame seems to have a beneficial effect in the treatment of moderate and severe periodontitis. This knowledge and the information that the intentional removal of tooth structure is not a requirement for periodontal healing and regeneration (Nyman et al. 1986, 1988, Goncalves et al. 2006), because bacterial lipopolysaccharide (LPS) is easily removed from the root surface (Hughes & Smales 1986, Moore et al. 1986), gave biological plausibility to the development of the full-mouth ultrasonic debridement protocol. The concept of periodontal debridement was suggested by Smart et al. (1990) as a conservative instrumentation regime of overlapping strokes and light pressure utilized for only a limited time period. The main goal here is to obtain a biocompatible root surface.

Although the possibility of treating chronic periodontitis with full-mouth ultrasonic debridement has been suggested (Koshy et al. 2005, Wennström et al. 2005, Zanatta et al. 2006), no information is available regarding the effects of this approach, with a maximum duration of 45 min., on the quantities of periodontopathogens and on host-immune inflammatory response. Thus, the aim of the present study was to clinically, microbiologically and immunologically characterize periodontal debridement as a therapeutic approach for severe chronic periodontitis, in comparison with the standard procedure.

## Material and Methods

### Study design

The present study was designed as a parallel, randomized, blinded and controlled clinical trial of 6 months duration to compare the clinical, microbiological and immunological outcomes after periodontal debridement (test group) with scaling and root planing (control group). The study design was, before commencement, approved by the Ethics Committee of the University of Campinas – UNICAMP (126/2005). All patients were individually informed about the nature of the proposed treatment, and informed consent forms were signed.

### Population screening

Twenty-seven subjects, from those referred for treatment to the Department of Prosthodontics and Periodontology in Piracicaba Dental School, University of Campinas – UNICAMP, Brazil, were recruited after a screening examination including a full medical and dental history, an intra-oral examination, a full-mouth periodontal probing and radiographic evaluation.

Subjects who were invited to participate met the following inclusion criteria: (1) diagnosis of severe chronic periodontitis by the presence of periodontal pockets with a clinical attachment loss of $\geqslant 5$ mm, bleeding on probing (BOP) and radiographic bone loss (Flemmig 1999); (2) at least eight teeth with a probing pocket depth (PPD) of $\geqslant 5$ mm and bleeding following pocket probing. At least two of the eight qualifying teeth must have PPD $\geqslant 7$ mm, and the pockets of a further two teeth must have PPD $\geqslant 6$ mm; (3) minimum of 20 teeth in both jaws (wisdom teeth excluded). Exclusion criteria were as follows: (1) periapical alterations on qualifying teeth; (2) medical disorders that require prophylactic antibiotic coverage or that could influence the response to treatment; (3) scaling and root planing in the preceding 6 months; (4) consumption of drugs known to affect periodontal status (antibiotic, anti-inflammatory, anticonvulsant, immunosuppressant and calcium channel blocker) within the past 6 months; (5) orthodontic therapy; (6) pregnancy and (7) smoking.

### Randomization and allocation concealment

Patients were randomized into two groups according to a computer-generated list. The allocation concealment was secured by having a person not involved in the study performing the randomization. This person was different from the one responsible for the treatment (S. B.) and different from the examiner (E. D. P. R.). The randomization code was not broken until all data had been collected. Thus, the treatment group was not revealed to the clinical examiner or to the statistician.

### Treatment

Patients initially received detailed information on the etiology of periodontal disease and instructions for proper, self-performed plaque control measures, including inter-dental cleaning with dental floss and inter-dental toothbrushes. In the initial sessions, patients also had plaque retentive factors (caries, excess of restorations and supragingival calculus) removed. The baseline measurements were done 30 days after this initial phase.

After random allocation, patients were subjected to one of two treatment groups: (1) control group – conventional quadrant-wise scaling and root planing, with an interval of 1 week between quadrants, using an ultrasonic scaler (Profi III, Dabi Atlante, Ribeirão Preto, São Paulo, Brazil) and Gracey curettes (Hu-Friedy, Chicago, IL, USA); (2) test group – one session of full-mouth periodontal debridement with a time limit of 45 min., using an ultrasonic scaler (Profi III). Specific tips were used (33 Probe, Amdent, Stockholm, Sweden). The control group did not receive a time limit for instrumentation, but 126 min. was the mean time taken to complete treatment. For both treatment protocols, local anesthesia was used as necessary. Only one clinician (S. B.) was responsible for administering the treatment throughout the study. This clinician was different from the calibrated examiner (E. D. P. R.) responsible for performing the clinical measurements. The examiner remained blinded throughout the study.

### Re-assessment examinations

After active treatment, all subjects were included in a maintenance programme composed of professional supragingival plaque control and reinforcement of oral hygiene instructions every month until the sixth month. At the third month recall visit, sites that exhibited PPD $\geqslant 5$ mm and bled on probing were re-instrumented. The maintenance programme also included an update of the medical and dental histories, extraoral and intra-oral soft tissue examination, dental examination and periodontal evaluation.

#### Clinical measurements

The following clinical parameters were taken at baseline and at 3 and 6 months after treatment. The presence of supragingival biofilm and gingival inflammation were evaluated by the visible plaque index (VPI) and the gingival bleeding index (GBI), dichotomously at six sites on all teeth in the mouth (Ainamo & Bay 1975). BOP was also measured dichotomously at six sites per tooth (Mühlemann & Son 1971). An individual occlusal stent was fabricated of self-curing clear resin to create fixed landmarks and to standardize the location and angulation of periodontal probes. The position of gingival margin (PGM) was measured from the stent to the gingival margin, and the relative attachment level (RAL) was measured from the stent to the bottom of periodontal pocket. The PPD was calculated based on RAL and PGM. The parameters VPI, GBI, BOP, PGM, RAL and PPD were measured using a standardized periodontal probe with 1 mm markings (PCPUNC 15® Hu-Friedy, Chicago, IL, USA).

Patients' perception of the treatment was also recorded, based on a self-filled questionnaire. Using a visual analogue scale from 0 to 10, the subjects were asked to mark the level of pain they experienced after treatment on the same day and on the next day. They were also instructed to record the number of analgesics taken after treatment and their body temperature, the evening after treatment and the morning of the next day, with a thermometer placed in the axilla. The subjects were also asked to report the incidence of oral ulcerations or other adverse effects. This questionnaire was given to the patient at the end of periodontal instrumentation. Thus, in the control group, the patients responded to the questionnaire only after the instrumentation of the last quadrant.

#### Examiner calibration

The investigator charged with clinical assessments was calibrated for intra-examiner repeatability before the start of the trial. Three patients with chronic periodontitis were enrolled for this purpose. Duplicate measurements ($N = 414$) for PPD and RAL were collected with an interval of 24 h between the first and second recording. The intra-class correlation coefficients, used as a measure of intra-examiner reproductibility, were 0.81 and 0.88 for mean PPD and RAL, respectively.

*Table 1.* Specific primers for the target microorganisms

| Bacteria | Primer 5′–3′ | Primer 3′–5′ |
|---|---|---|
| Pg | CATAGATATCACGAGGAACTCCGATT | AAACTGTTAGCAACTACCGATGTGG |
| Tf | CGTTTCCGAAGAGTATAACCACA | CATGCAGCTTGATATTCTGAGG |
| Aa | GAACCTTACCTACTCTTGACATCCGAA | TGCAGCACCTGTCTCAAAGC |

Pg, *Porphyromonas gingivalis;* Aa, *Aggregatibacter actinomycetemcomitans*; Tf, *Tannerella forsythia*.

#### Sample collection

Subgingival biofilm samples were collected from one pocket with a PPD of 5 mm and from another pocket with a PPD of 7 mm, at baseline, and at 3 and 6 months after treatment. Following the careful removal of supragingival biofilm, areas were washed with a water spray, isolated with cotton rolls and gently dried. A sterile paper point (No: 35) was inserted into the bottom of the periodontal pocket for 30 s. The paper points were placed into sterile tubes containing 300 $\mu$l of reduced transport fluid (Syed & Loesche 1972).

Gingival crevicular fluid (GCF) was collected from the same sites of microbiological sampling. After subgingival biofilm collection, teeth were washed again, and the area was isolated and gently dried. GCF was collected by placing filter paper strips (Periopaper, Oraflow, Plainview, NY, USA) into the pocket until a slight resistance was perceived, and then left in place for 15 s. Immediately, the volume of the sample was measured with the aid of a calibrated electronic gingival fluid measuring device (Periotron 8000, Oraflow, Plainview, NY, USA). After volume measurements, the strips were placed into sterile tubes containing 400 $\mu$l of phosphate-buffered saline (PBS) with 0.05% Tween-20 (Gamonal et al. 2000). Strips contaminated by visible blood were discarded.

All samples (subgingival biofilm and GCF) were immediately stored at $-20^{\circ}$C. Only one examiner, the same one charged with clinical measures, collected all microbial and GCF samples.

#### Microbiological evaluation

Real-time PCR detection and quantification were based on the amplification of variable regions of the 16S rRNA genes of *Porphyromonas gingivalis* (Pg), *Aggregatibacter actinomycetemcomitans* (Aa) and *Tannerella forsythia* (Tf). The primer sequences of Pg and Aa were based on the reports by Kozarov et al. (2006) and Boutaga et al. (2005), respectively. Primers for Tf were designed with the use of the LightCycler probe design software (Roche Diagnostics GmbH, Mannheim, Germany). The primers used in the present study are listed in Table 1.

Bacterial DNA was extracted from subgingival biofilm, as described previously (Saito et al. 2006). Reaction efficiency was optimized, and primer final concentrations of 0.5 $\mu$M for Pg and Aa and 0.3 $\mu$M for Tf were chosen. Real-time PCR was performed in the LightCycler system (Roche Diagnostics GmbH) with the FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmbH). For each run, water was used as the negative control. Briefly, amplification was performed in a 10 $\mu$L final volume containing 2.5 $\mu$l of template DNA. The concentration of the DNA used in each run was always 50 $\mu$g/ml (Teo et al. 2002). The amplification profiles were as follows: 95/10, 55/5, 72/4 [temperature ($^{\circ}$C)/time (seconds)] and 40 cycles for Pg; 95/10, 46/5, 72/5 and 45 cycles for Tf; 95/10, 55/5, 72/3 and 40 cycles for Aa. Melting peaks were used to determine the specificity of the PCR.

Absolute quantification of target bacteria in clinical samples was performed using Pg (ATCC 33277), Tf (ATCC 43037) and Aa (JP2) as controls. Standard curves were made with these controls. The standard curves were used to convert cycle threshold scores into the number of bacterial cells using controls with known amounts of bacterial-specific DNA. The level of detection was set to a $10^3$ bacteria/plaque sample for all target bacteria. The determination of DNA content in controls was based on the genome size of each bacteria and the mean weight of one nucleotide pair (Dolezel et al. 2003).

#### Immunological evaluation

Aliquots of each GCF sample were assayed by an enzyme-linked

immunosorbent assay (ELISA) using commercially available kits (R&D System Inc, MN, USA) for human prostaglandin $E_2$ ($PGE_2$), interleukin-1 beta (IL-1$\beta$), interferon-gamma (IFN-$\gamma$) and interleukin-10 (IL-10), according to the manufacturer's instructions. Previously, samples were diluted with the diluent of the kit. The dilution was considered to calculate the concentration of each GCF substance. This concentration was calculated with a standard curve prepared by using the standard proteins in the kit. The prepared measurement range of the standard curve for $PGE_2$ was 125–19.26 pg/ml; for IL-1$\beta$ the range was 8–0.125 pg/ml; for IFN-$\gamma$ the range was 1000–15.60 pg/ml and for IL-10 it was 50–0.78 pg/ml. The ELISA assays were run in duplicate, and mean values were used to calculate concentrations of each cytokine.

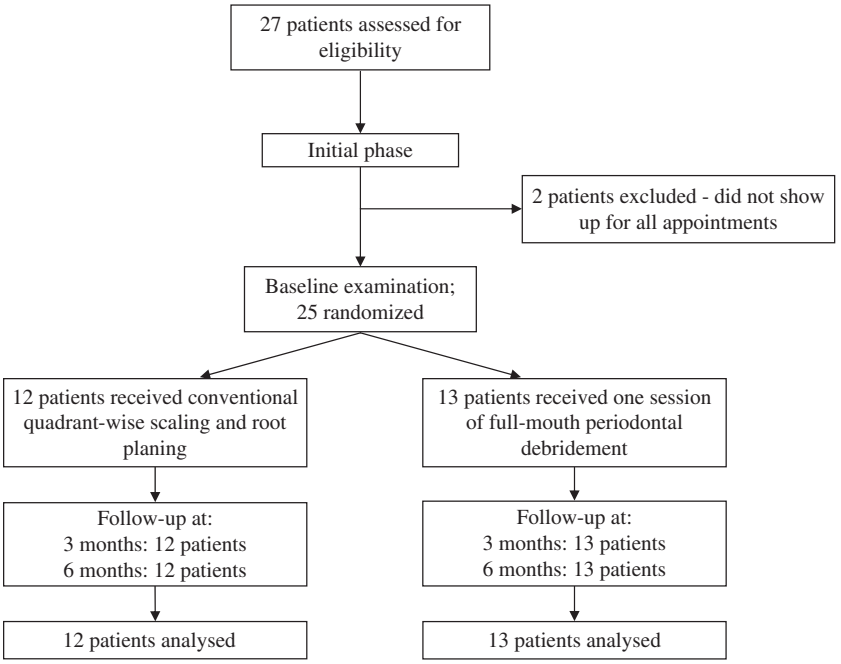


*Fig. 1.* Flowchart for study patients.

#### Power calculation

Power calculation was done before the study with an SAS 9.01 programme (Release 9.1, 2003, SAS Institute Inc., Cary, NC, USA). This analysis indicated that with 12 subjects in each group the study would have $>80\%$ power to detect a 1 mm difference in clinical attachment level between the two groups. After the completion of the study, considering the SD of each group of the present clinical trial, the power value was confirmed to be $>80\%$ to detect a 1 mm difference in clinical attachment level between the two groups.

#### Data management and statistical analysis

Repeated measures analysis of variance (ANOVA) was used to detect intra- and intergroup differences in VPI. The Friedman test was used to detect intragroup differences in GBI, BOP, PGM, PPD, RAL, bacterial counts and cytokine levels among all periods, and the Mann–Whitney test was used to detect intergroup differences of these parameters at each time interval. The Wilcoxon test was used to compare moderate and deep pockets in each group. Mean and SD were calculated for each patient. In the microbiological evaluation, when the PCR did not detect bacteria, the value for statistical analysis was zero. The proportion of sites presenting a RAL gain of $\geqslant 2$ mm and the number of sites re-treated at 3 and 6 months were compared between groups with $\chi^2$ analysis. All evaluations used the subject as the unit of measurement. Individual sites were only compared regarding the RAL gain of $\geqslant 2$ mm, the occurrence of BOP and the number of sites referred for treatment. The level of pain experienced after treatment and the number of analgesics taken after treatment were compared between groups with the Mann–Whitney test. The occurrence of fever and oral ulceration was compared with Fisher's exact test. All analysis was carried out with SAS Software 2001-Release 8.2 (SAS Institute Inc., Cary, NC, USA). An experimental level of significance ($\alpha$) was set at 0.05. RAL was considered as the primary outcome variable. All other parameters were considered secondary outcomes.

## Results

#### Subject accountability

Two patients did not show up for all the appointments, for reasons not related to the study; thus, a total of 25 patients completed the study (12 in the control group and 13 in the test group). Figure 1 illustrates the study flowchart. The number of sites showing a baseline PPD of $\geqslant 5$ mm and BOP (qualifying sites) were 104 in the control group and 108 in the test group, out of which 26.9% and 19.4%, respectively, had a PPD of $\geqslant 7$ mm. Data analysis at baseline indicated that experimental groups were balanced for age, gender and clinical parameters (Table 2).

#### Study schedule

Subject recruitment started in July 2005 and was completed by the end of June 2006. All the 6-month follow-up visits were completed in December 2006. Laboratory analysis was completed in August 2007. Data entry of all information and statistical analysis were performed by the end of August 2007.

#### Clinical results

##### *VPI, GBI and BOP*

The oral hygiene status during the course of the study is illustrated in Fig. 2a. No significant difference between groups was observed at any of the examination intervals. Regarding GBI, also no significant difference was observed between groups at any experimental period, whereas both groups had a significant reduction at 3 and 6 months when compared with baseline ($p<0.05$). The marginal gingival bleeding during the course of the study is shown in Fig. 2b. BOP was evaluated specifically on qualifying sites. This analysis

*Table 2.* Baseline means (± SD) of age, gender and clinical parameters*

| Characteristic | Control group | | Test group | |
|---|---|---|---|---|
| Age (mean/range) | 38.9 (33–55) | | 45.5 (30–66) | |
| % Males | 25.0 | | 30.8 | |
| VPI (%)† | 40.26 (± 16.66) | | 30.48 (± 12.77) | |
| GBI (%)† | 20.45 (± 8.22) | | 14.65 (± 6.88) | |
| BOP (%)† | 26.15 (± 10.82) | | 29.78 (± 13.45) | |
| | Moderate pockets | Deep pockets | Moderate pockets | Deep pockets |
| PGM (mm) | 2.28 (± 1.24) | 1.69 (± 1.02) | 2.14 (± 0.88) | 2.09 (± 0.82) |
| PPD (mm) | 5.44 (± 0.19) | 7.47 (± 1.65) | 5.47 (± 0.23) | 7.33 (± 1.54) |
| RAL (mm) | 7.72 (± 1.23) | 9.16 (± 1.91) | 7.61 (± 0.94) | 9.42 (± 1.92) |

*At baseline, no significant differences were noted in the demographic and clinical parameters between groups (analysis by a non-paired *t*-test for age, by repeated measures ANOVA for VPI and by Mann–Whitney test for the other clinical parameters).

†Values of VPI, GBI and BOP refer to means of the whole mouth, while values of PGM, PPD and RAL refer to means of the qualifying sites.

VPI, visible plaque index; GBI, gingival bleeding index; BOP, bleeding on probing; PGM, position of gingival margin; PPD, probing pocket depth; RAL, relative attachment level.

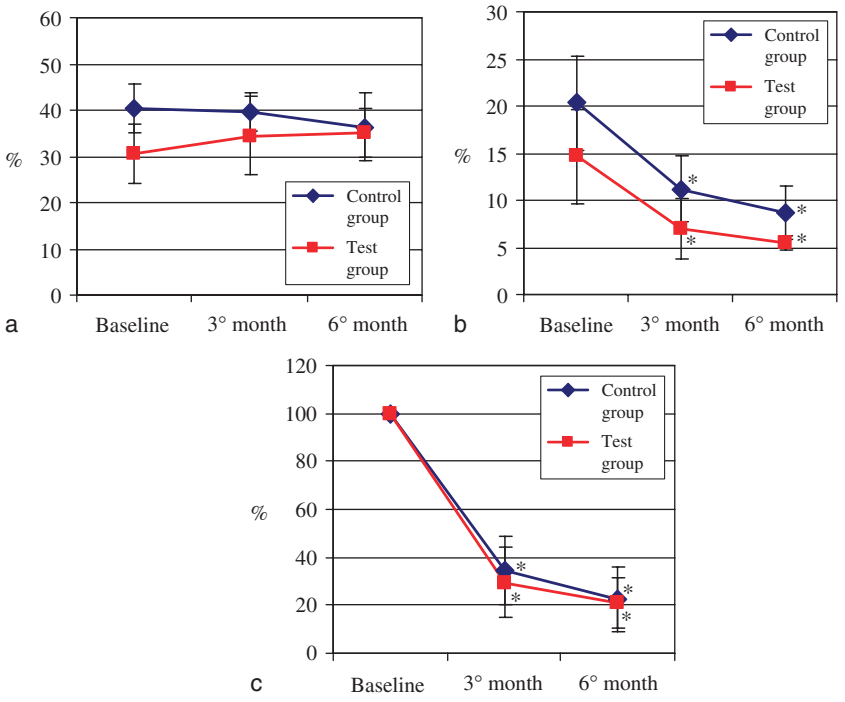


*Fig. 2.* Clinical parameters at different time intervals for control and test groups. (a) VPI. Analysis between groups by repeated measures ANOVA. (b) GBI. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (c) BOP. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test).

showed that BOP was reduced over time in both groups, but no significant difference was found between them (Fig. 2c).

### *PGM, PPD and RAL*

Both groups showed, at 3 and 6 months, an increase in PGM, a reduction in PPD and a gain in RAL (Table 3). The increase in PGM reflects the apical migration of gingival margin after treatment. At 6 months, the mean decrease in PPD was $2.93 \pm 0.33$ and $2.45 \pm 0.50$ mm in the control and test groups, respectively ($p>0.05$). The mean gain in RAL was $1.87 \pm 1.13$ and $1.63 \pm 0.85$ mm in the control and test groups, respectively ($p>0.05$). The results of PGM, PPD and RAL strictly refer to the qualifying sites.

There was no difference between groups regarding the proportions of sites presenting a RAL gain of $\geqslant 2$ mm at any experimental time. At 3 months, the control group had 48.08% of sites showing a RAL gain of 2 mm or more, and the test group had 44.44%. These values were 56.73% (control group) and 43.52% (test group) at 6 months. The percentage of sites that needed re-treatment at 3 months was 9.61% in the control group and 10.18% in the test group ($p>0.05$). At 6 months, the values were 4.81% and 9.26% ($p>0.05$), for the control and test groups, respectively.

### *Treatment discomfort*

The degree of treatment discomfort following treatment revealed no difference between the two treatment approaches. In addition, no difference was observed between groups with regard to the ingestion of analgesics, and the occurrence of fever and ulcerations (Table 4). None of the patients reported acute problems (e.g. periodontal abscesses) during the study period.

### Microbiological results

In the baseline samples, Pg was the most frequent microorganism found in subgingival biofilm (72.50% in the control group and 73.07% in the test group), whereas Aa had the lowest frequency of detection (40.83% in the control group and 42.15% in the test group). The Tf frequency of detection at baseline was 45.83% in the control group and 69.23% in the test group. An intergroup analysis demonstrated no significant difference between groups for any of the species assessed, at any time period ($p>0.05$). Intra-group analyses showed that the reduction, 3 and 6 months after treatment, in these detection frequencies was statistically significant for Pg in both groups and for Tf, only in the test group (Fig. 3).

The intergroup quantitative analysis of the three periodontopathogens showed, as described in the frequency analysis, no difference between groups. At 6 months, the intra-group comparison showed a significant reduction in the amounts of Pg in both groups and of Tf, only in the test group. With respect to Aa, none of the treatment modalities produced a statistically significant reduction in the amount of this pathogen, although for both groups a slight

*Table 3*. Position of gingival margin (PGM) increase, probing pocket depth (PPD) reduction and relative attachment level (RAL) gain (mm) in initially moderate and deep pockets at different time intervals for control and test groups*

| | Moderate pockets | | | | Deep pockets | | | |
|---|---|---|---|---|---|---|---|---|
| | control group | | test group | | control group | | test group | |
| | mean ± SD | median (range) | mean ± SD | median (range) | mean ± SD | median (range) | mean ± SD | median (range) |
| PGM | | | | | | | | |
| 3 months | 0.75 ± 0.71 | 0.66 (0.8–1.4) | 0.68 ± 0.77 | 0.63 (0.1–1.5) | 1.33 ± 1.10 | 1.27 (1.0–2.0) | 1.11 ± 0.82 | 1.00 (0.7–2.0) |
| 6 months | 0.75 ± 0.79 | 0.69 (0.0–1.2) | 0.72 ± 0.69 | 0.63 (0.0–1.5) | 1.54 ± 1.00 | 1.67 (1.0–2.0) | 1.03 ± 0.87 | 1.00 (0.5–1.5) |
| PPD | | | | | | | | |
| 3 months | 2.02 ± 0.77 | 2.16 (0.2–3.0) | 1.81 ± 0.59 | 1.58 (1.1–3.1) | 3.51 ± 0.58† | 3.45 (2.7–5.0) | 3.31 ± 0.88‡ | 3.00 (2.5–4.5) |
| 6 months | 2.27 ± 0.43 | 2.41 (1.7–3.0) | 1.93 ± 0.62 | 2.00 (1.2–3.1) | 3.94 ± 0.49† | 3.88 (3.3–5.0) | 3.44 ± 0.76‡ | 3.50 (2.5–4.5) |
| RAL | | | | | | | | |
| 3 months | 1.27 ± 1.30 | 1.38 (0.0–2.0) | 1.13 ± 0.99 | 1.13 (0.5–1.9) | 2.18 ± 0.85† | 2.10 (1.0–4.0) | 2.20 ± 0.98‡ | 2.00 (1.5–3.0) |
| 6 months | 1.52 ± 1.46 | 1.52 (0.9–2.5) | 1.21 ± 0.98 | 1.22 (0.6–2.1) | 2.40 ± 1.04† | 2.00 (1.5–4.0) | 2.41 ± 0.92‡ | 2.25 (1.5–3.0) |

*No significant differences were noted between control and test sites either in moderate or deep pockets (analysis by Mann–Whitney test).
†Difference ($p<0.05$) from moderate pockets of the control group (analysis by Wilcoxon's test).
‡Difference ($p<0.05$) from moderate pockets of the test group (analysis by Wilcoxon's test).
PGM, position of gingival margin; PPD, probing pocket depth; RAL, relative attachment level.

*Table 4*. Patients' perception of treatment*

| Treatment effects | Control group | Test group |
|---|---|---|
| Level of pain,† day 0 | 2.50 (0–5) | 2.42 (0–6) |
| Level of pain,† day 1 | 0.67 (0–2) | 1.19 (0–6) |
| No. of analgesics,† day 0 | 0.33 (0–1) | 0.53 (0–2) |
| No. of analgesics,† day 1 | 0.0 (0–0) | 0.23 (0–2) |
| Fever, day 0 | 1/12 | 1/13 |
| Fever, day 1 | 0/12 | 0/13 |
| Oral ulceration | 2/12 | 2/13 |

*No significant differences were noted between control and test groups (analysis by Mann–Whitney test for level of pain and number of analgesics and by Fisher's exact test for occurrence of fever and oral ulceration).
†Mean (minimum – maximum).
Day 0: same day after treatment; day 1: next day after treatment.

tendency towards reduction was observed, with a $p$ value of 0.07 for the test group at 6 months (Fig. 4).

#### Immunological results

An intergroup analysis demonstrated no significant difference between groups in GCF volume, at all periods of evaluation. However, the intra-group analysis showed that, for both groups, the reduction after treatment was statistically significant at the third month (data not shown).

No difference was observed between control and test groups in the quantity of $PGE_2$, IL-1$\beta$, IFN-$\gamma$ and IL-10 in GCF, at all periods of evaluation (Fig. 5). Only the test therapy promoted, at the sixth month, a statistically significant reduction in the levels of IL-1$\beta$. Furthermore, an elevation in the levels of IL-10 was observed at 3 and 6 months after therapy, only in the test group.

## Discussion

Non-surgically performed scaling and root planing (SRP) is an effective treatment modality for periodontal disease; however, concern exists regarding microbial recolonization and the level of instrumentation required for periodontal healing (Kinane 2005). In this context, with periodontal debridement, the entire dentition is instrumented during a single session for a restricted period of time. Thus, the objective of the present study was to evaluate the clinical, microbiological and immunological effects of periodontal debridement in the treatment of severe chronic periodontitis.

The present study demonstrated that, in patients with severe chronic periodontitis, the periodontal debridement resulted in clinical improvements similar to those achieved with the traditional approach of quadrant-wise scaling and root planing. The clinical results were reinforced by the micro and immunological results. No difference was observed between treatments in the prevalence and quantity of Pg, Tf and Aa. In addition, no difference was observed between groups regarding the levels of $PGE_2$, IL-1$\beta$, IFN-$\gamma$ and IL-10 in GCF.

The magnitude of the clinical improvements was similar to the previously published data. Six months after SRP, the reduction in PPD was 2.27 and 3.94 mm for initially moderate and deep pockets, respectively. The gain in RAL was 1.52 and 2.40 mm for initially moderate and deep pockets, respectively. Badersten et al. (1981) reported a PPD reduction of 1.30–1.70 mm in moderate pockets and of 2.30–3.00 mm in deep pockets. Cobb (1996) reported a gain of attachment in moderate pockets of 0.55 mm and in deep pockets of 1.19 mm.

Six months after periodontal debridement, the PPD reduction was 1.93 and 3.44 mm for initially moderate and deep

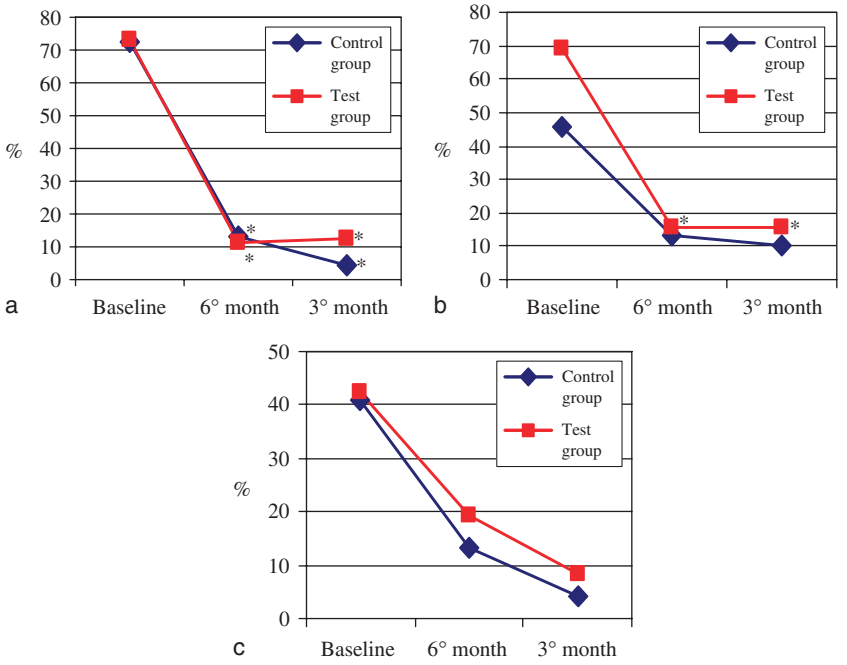


*Fig. 3.* Detection frequency of periodontal pathogens in subgingival biofilm at different time intervals for control and test groups. (a) *P. gingivalis*. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (b) *T. forsythia*. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (c) *A. actinomycetemcomitans*.

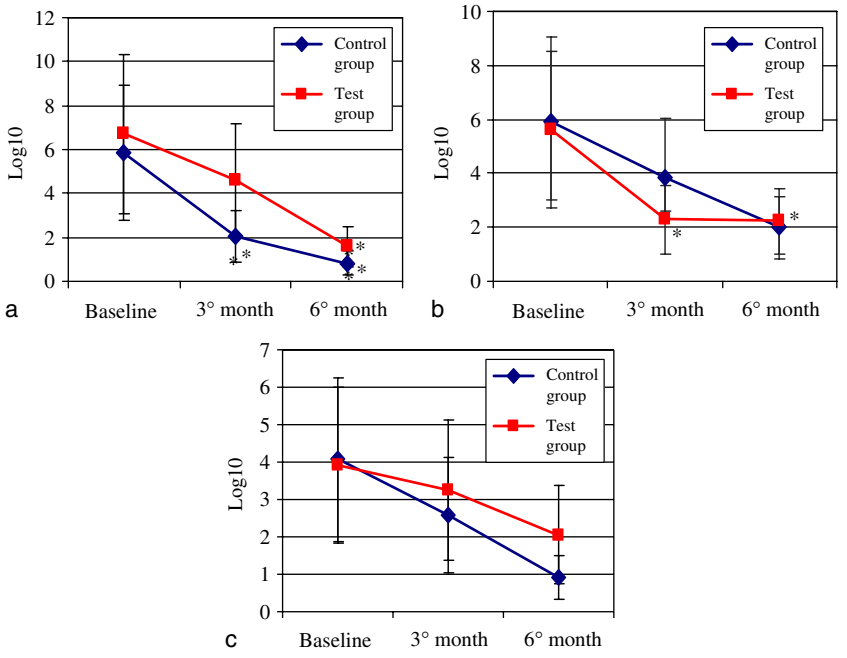


*Fig. 4.* Amounts ($\log_{10}$) of periodontal pathogens in subgingival biofilm at different time intervals for control and test groups. (a) *P. gingivalis*. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (b) *T. forsythia*. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (c) *A. actinomycetemcomitans*.

pockets, respectively. The RAL gain was 1.21 and 2.41 mm for initially moderate and deep pockets, respectively. Wennström et al. (2005) showed a PPD change of 1.8 and 2.9 mm and a RAL gain of 1.3 and 2.2 mm for moderate and deep pockets, respectively.

The percentage of sites showing a RAL gain of $\geqslant 2$ mm and no need of re-treatment confirmed the good results obtained with both treatment strategies. These are important parameters to evaluate the clinical significance of a treatment outcome (Killoy 2002). At 3 months, the highest need for re-treatment was 10.18%. This value is markedly different from those published by Wennström et al. (2001), where all sites with a PPD of $\geqslant 5$ mm (42%) in the debridement group were qualified as needing re-treatment. This difference is probably explained by the fact that, in the present study, only sites with a PPD of $\geqslant 5$ mm and BOP were re-treated. This decision was taken because BOP is an important outcome measurement, since the absence of BOP in recall patients has been associated with clinical stability over time (Lang et al. 1990). Furthermore, BOP associated with PPD has shown the greatest diagnostic accuracy for future attachment loss (Vanooteghem et al. 1987).

Real-time PCR was used for the detection and quantification of periodontal pathogens in this study. PCR is a rapid and sensitive method for the detection of bacterial DNA sequences, and the quantitative analysis is important for determining the actual impact of the treatment protocols on the bacterial load, especially when the concept of a threshold number of bacteria is considered (Doungudomdacha et al. 2001, Mackay 2004). With this concept, in the present study, the observation of a marked reduction in the prevalence and number of Pg in both groups shows that periodontal debridement is as efficient as SRP in the control of this pathogen (Takamatsu et al. 1999, Jervoe-Storm et al. 2007).

The reduction of Tf after SRP does not appear to be as simple (Darby et al. 2001, Jervoe-Storm et al. 2007). In the present study, the reduction in the prevalence and counts of Tf was only observed in the test group. The reductions observed in the counts of Pg and Tf were observed at the third month of evaluation and maintained throughout the study. Thus, periodontal maintenance of monthly returns achieved its purpose, permitting the proper evaluation of subgingival therapy and contributing to the good results observed, 6 months after therapy, in both groups.

In relation to Aa, a small number of sites and patients positive for this microorganism were detected at baseline. This may explain why the reductions observed after treatments were not statistically significant. Cugini et al.

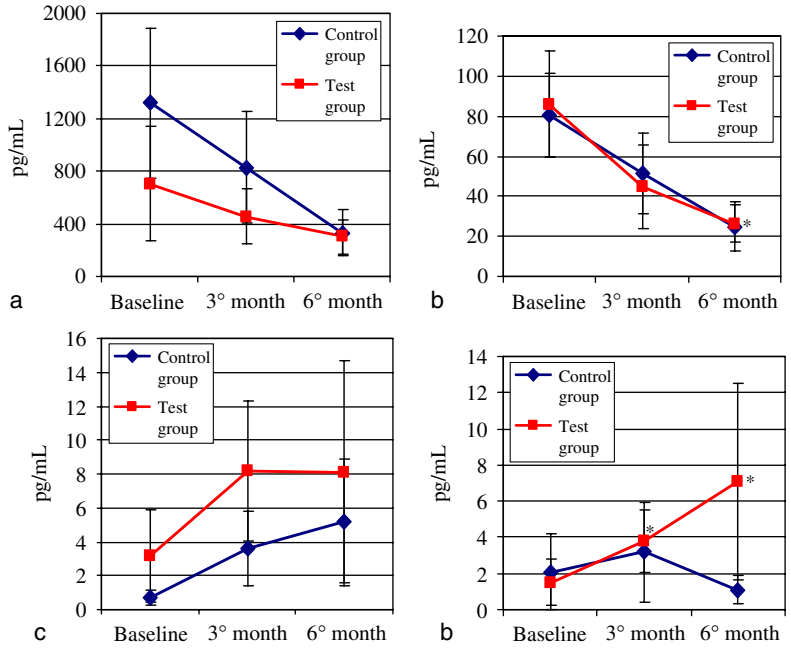


*Fig. 5.* Amounts (pg/ml) of cytokines in GCF at different time intervals for control and test groups. (a) $PGE_2$. (b) IL-1$\beta$. *Significant reduction ($p<0.05$) from baseline (analysis by Friedman test). (c) IFN-$\gamma$. (d) IL-10. *Significant elevation ($p<0.05$) from baseline (analysis by Friedman test).

(2000), also presenting a low baseline frequency of positive patients, did not observe an effect of SRP on Aa levels. This and other comparisons should be interpreted with caution because the microbiological observations are influenced by the technique used for the detection of microorganisms and by the studied population, because there are marked differences in the microbial profiles of subgingival biofilm from chronic periodontitis patients in different countries (Haffajee et al. 2004).

Immunological evaluation is important because the balance between local levels of cytokines, stimulated in response to periodontopathogenic bacteria and their products, determines the outcome of the immune response. This means that cytokines play an important role in the initiation and progression of periodontitis. $PGE_2$ has been implicated as a key inflammatory mediator in periodontal disease, causing decreased collagen synthesis and stimulating osteoclastic bone resorption (Dziak 1993, Arai et al. 1995). Thus, one of the results of periodontal therapy should be the reduction in the levels of this mediator (Alexander et al. 1996). However, in the present study, inter-individual variability could have prevented the detection of statistical significance in the reductions observed.

IL-1$\beta$ is another potent proinflammatory cytokine involved in the pathogenesis of periodontal disease (Goutoudi et al. 2004), the reduction of which is also desired after treatment. This was observed only in the test group and may indicate the presence of a subclinical inflammation, after treatment, in the control group, because the levels of IL-1$\beta$ in GCF seem more effective than clinical parameters for evaluating subgingival inflammation (Yoshinari et al. 2004).

Like IL-1$\beta$, IFN-$\gamma$ is also involved in the Th1 immune response and induces mainly a cell-mediated response. Little is known about the role of this cytokine in periodontitis, but it seems to increase the risk of disease progression (Alpagot et al. 2003). The desired reduction after periodontal therapy was not observed in the present study. A partial explanation for this observation was the low frequency of detection and low concentration of this cytokine, which can be attributed to the sensitivity of the immunoassay used.

In contrast, IL-10 is involved in Th2 immune responses, promotes humoral immunity due to the production of B cell growth and has been implicated in the suppression of tissue destruction (Moore et al. 1993). Thus, following periodontal treatment an increase in the concentration of IL-10 is desired (Górska et al. 2003). In the present study, an increase in the levels of IL-10 in GCF was observed only in the test group.

This information, and the fact that the reduction of IL-1$\beta$ observed in the present study was statistically significant only in the test group, could be associated with the fact that a reduction in the counts of Tf was also observed only in the test group. Although no difference was observed between groups, these results could predispose the control group to greater disease recurrence; however, only a longitudinal evaluation of the patients could answer this question (Tomasi et al. 2006).

In the present study, all clinical, microbiological and immunological analyses were coherent because no statistical differences were observed between groups. These data may be interpreted as favourable for the use of periodontal debridement, because the reduction in instrumentation time and, probably, the reduced loss of tooth structure are clinically important achievements for the patient and for the long-term maintenance of the tooth. It is important to note that this is true for the sample analyzed and cannot be extrapolated for aggressive periodontitis, smokers and patients with important systemic diseases.

## Conclusion

Periodontal debridement resulted in a similar clinical, microbiological and immunological outcome when compared with standard scaling and root planing and therefore may be assumed as a viable approach to deal with severe chronic periodontitis.

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## References

Ainamo, J. & Bay, I. (1975) Problems and proposals for recording gingivitis and plaque. *International Dental Journal* **25**, 229–235.

Alexander, D. C. C., Martin, J. C., King, P. J., Powell, J. R., Caves, J. & Cohen, M. E. (1996) Interleukin-1 beta, prostaglandin $E_2$, and immunoglobulin G subclasses in gingival crevicular fluid in patients undergoing periodontal therapy. *Journal of Periodontology* **67**, 755–762.

Alpagot, T., Font, K. & Lee, A. (2003) Longitudinal evaluation of GCF INF-γ levels and periodontal status in $HIV^+$ patients. *Journal of Clinical Periodontology* **30**, 944–948.

Arai, H., Nomura, Y., Kinoshita, M., Shimizu, H., Ono, K., Goto, H., Takigawa, M., Nishimura, F., Washio, N., Kurihara, H. & Murayama, Y. (1995) Response of human gingival fibroblasts to prostaglandins. *Journal of Periodontal Research* **30**, 303–311.

Badersten, A., Nilveus, R. & Egelberg, J. (1981) Effect of nonsurgical therapy I. Moderately advanced periodontitis. *Journal of Clinical Periodontology* **8**, 57–72.

Badersten, A., Nilveus, R. & Egelberg, J. (1984) Effect of nonsurgical periodontal therapy. II. Severely advanced periodontitis. *Journal of Clinical Periodontology* **11**, 63–76.

Boutaga, K., van Winkelhoff, A. J., Vandenbroucke-Grauls, C. M. J. E. & Savelkoul, P. H. M. (2005) Periodontal pathogens: a quantitative comparison of anaerobic culture and real-time PCR. *FEMS Immunology & Medical Microbiology* **45**, 191–199.

Cobb, C. M. (1996) Non-surgical pocket therapy: mechanical. *Annals of Periodontology* **1**, 443–490.

Cugini, M. A., Haffajee, A. D., Smith, C., Kent, R. L. Jr. & Socransky, S. S. (2000) The effect of scaling and root planning on the clinical and microbiological parameters of periodontal diseases: 12-month results. *Journal of Clinical Periodontology* **27**, 30–36.

Darby, I. B., Mooney, J. & Kinane, D. F. (2001) Changes in subgingival microflora and humoral immune response following periodontal therapy. *Journal of Clinical Periodontology* **28**, 796–805.

Dolezel, J., Bartos, J., Voglmayr, H. & Greilhuber, J. (2003) Nuclear DNA content and genome size of trout and human. *Cytometry A* **51**, 127–128.

Doungudomdacha, S., Rawlinson, A., Walsh, T. F. & Douglas, C. W. I. (2001) Effect of nonsurgical periodontal treatment on clinical parameters and the number of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* at adult periodontitis sites. *Journal of Clinical Periodontology* **28**, 437–445.

Dziak, R. (1993) Biochemical and molecular mediators of bone metabolism. *Journal of Periodontology* **64**, 407–415.

Flemmig, T. F. (1999) Periodontitis. *Annals of Periodontology* **4**, 32–38.

Gamonal, J., Acevedo, A., Bascones, A., Jorge, O. & Silva, A. (2000) Levels of interleukin-1β, -8 and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *Journal of Periodontology* **71**, 1535–1545.

Goncalves, P. F., Gurgel, B. C., Pimentel, S. P., Sallum, E. A., Sallum, A. W., Casati, M. Z. & Nociti, F. H. Jr. (2006) Effect of two different approaches for root decontamination on new cementum formation following guided tissue regeneration: a histomorphometric study in dogs. *Journal of Periodontal Research* **41**, 535–540.

Górska, R., Gregorek, H., Kowalski, J., Laskus-Perendyk, A., Syczewska, M. & Madalinski, K. (2003) Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. *Journal of Clinical Periodontology* **30**, 1046–1052.

Goutoudi, P., Diza, E. & Arvanitidou, M. (2004) Effect of periodontal therapy on crevicular fluid interleukin-1β and interleukin-10 levels in chronic periodontitis. *Journal of Dentistry* **32**, 511–520.

Haffajee, A. D., Bogren, A., Hasturk, H., Feres, M., Lopez, N. J. & Socransky, S. S. (2004) Subgingival microbiota of chronic periodontitis subjects from different geographic locations. *Journal of Clinical Periodontology* **31**, 996–1002.

Hughes, F. J. & Smales, F. C. (1986) Immunohistochemical investigation of the presence and distribution of cementum-associated lipopolysaccharides in periodontal disease. *Journal of Periodontal Research* **21**, 660–667.

Jervoe-Storm, P. M., AlAhdab, H., Semman, E., Fimmers, R. & Jepsen, S. (2007) Microbiological outcomes of quadrant versus full-mouth root planing as monitored by real-time PCR. *Journal of Clinical Periodontology* **34**, 156–163.

Killoy, W. J. (2002) The clinical significance of local chemotherapies. *Journal of Clinical Periodontology* **29**, 22–29.

Kinane, D. F. (2005) Single-visit, full-mouth ultrasonic debridement: a paradigm shift in periodontal therapy? *Journal of Clinical Periodontology* **32**, 732–733.

Koshy, G., Kawashima, Y., Kiji, M., Nitta, H., Umeda, M., Nagasawa, T. & Ishikawa, I. (2005) Effects of single-visit full-mouth ultrasonic debridement versus quadrant-wise ultrasonic debridement. *Journal of Clinical Periodontology* **32**, 734–743.

Kozarov, E., Sweier, D., Shelburne, C., Progulske-Fox, A. & Lopatin, D. (2006) Detection of bacterial DNA in atheromatous plaques by quantitative PCR. *Microbes and Infection* **8**, 687–693.

Lang, N. P., Adler, R., Joss, A. & Nyman, S. (1990) Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* **17**, 714–721.

Mackay, I. M. (2004) Real-time PCR in the microbiology laboratory. *Clinical Microbiology and Infection* **10**, 190–212.

Moore, J., Wilson, M. & Kieser, J. B. (1986) The distribution of bacterial lipopolysaccharide (endotoxin) in relation to periodontally involved root surfaces. *Journal of Clinical Periodontology* **13**, 748–751.

Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P. & Mosmann, T. R. (1993) Interleukin 10. *Annual Review of Immunology* **11**, 165–71.

Mühlemann, H. R. & Son, S. (1971) Gingival sulcus bleeding-a leading symptom in initial gingivitis. *Helvetica Odontologica Acta* **15**, 107–113.

Nyman, S., Sahed, G., Ericsson, I., Gottlow, J. & Karring, T. (1986) Role of ''diseased'' root cementum in healing following treatment of periodontal disease. An experimental study in the dog. *Journal of Periodontal Research* **21**, 496–503.

Nyman, S., Westfelt, E., Sahed, G. & Karring, T. (1988) Role of ''diseased'' root cementum in healing following treatment of periodontal disease. A clinical study. *Journal of Clinical Periodontology* **15**, 464–468.

O'Leary, T. J. (1986) The impact of research on scaling and root planing. *Journal of Periodontology* **57**, 69–75.

Quirynen, M. & Bollen, C. M. L. (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *Journal of Clinical Periodontology* **22**, 1–14.

Quirynen, M., Mongardini, C., De Soete, M., Pauwels, M., Coucke, W., Van Eldere, J. & van Steenberghe, D. (2000) The role of chlorhexidine in the one-stage full-mouth disinfection. Long-term clinical and microbiological observations. *Journal of Clinical Periodontology* **27**, 578–589.

Quirynen, M., De Soete, M., Boschmans, G., Pauwels, M., Coucke, W., Teughels, W. & van Steenberghe, D. (2006) Benefit of ''one-stage full-mouth disinfection'' is explained by disinfection and root planing within 24 hours: a randomized controlled trial. *Journal of Clinical Periodontology* **33**, 639–647.

Saito, D., Leonardo, R. T., Rodrigues, J. L., Tsai, S. M., Höfling, J. F. & Gonçalves, R. B. (2006) Identification of bacteria in endodontic infections by sequence analysis of 16S rDNA clone libraries. *Journal of Medical Microbiology* **55**, 101–107.

Smart, G. J., Wilson, M., Davies, E. H. & Kieser, J. B. (1990) Assessment of ultrasonic root surface debridement of residual endotoxin levels. *Journal of Clinical Periodontology* **1**, 174–178.

Syed, S. A. & Loesche, W. J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–644.

Takamatsu, N., Yano, K., He, T., Umeda, M. & Ishikawa, I. (1999) Effect of initial periodontal therapy on the frequency of detection *Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*. *Journal of Periodontology* **70**, 574–580.

Teo, I. A., Choi, J. W., Morlese, J., Taylor, G. & Shaunak, S. (2002) LightCycler qPCR optimization for low copy number target DNA. *Journal of Immunological Methods* **270**, 119–133.

Tomasi, C., Bertelle, A., Dellasega, E. & Wennström, J. L. (2006) Full-mouth ultrasonic debridement and risk of disease recurrence:

a 1-year follow-up. *Journal of Clinical Periodontology* **33**, 626–631.

Vanooteghem, R., Hutchens, L. H., Garrett, S., Kiger, R. & Egelberg, J. (1987) Bleeding on probing and probing depth as indicators of the response to plaque control and root debridement. *Journal of Clinical Periodontology* **14**, 226–230.

Wennström, J. L., Newman, H. N., MacNeill, S. R., Killoy, W. J., Griffiths, G. S., Gillam, D. G., Krok, L., Needleman, I. G., Weiss, G. & Garrett, S. (2001) Utilization of locally delivered doxycycline in non-surgical treatment of chronic periodontitis. A comparative multicenter trial of 2 treatment approaches. *Journal of Clinical Periodontology* **28**, 753–761.

Wennström, J. L., Tomasi, C., Bertelle, A. & Dellasega, E. (2005) Full-mouth ultra-sonic debridement versus quadrant scaling and root planing as an initial approach in the treatment of chronic periodontits. *Journal of Clinical Periodontology* **32**, 851–859.

Yoshinari, N., Kawase, H., Mitani, A., Ito, M., Sugiishi, S., Matsuoka, M., Shirozu, N., Ishihara, Y., Bito, B., Hiraga, M., Arakawa, K. & Noguchi, T. (2004) Effects of scaling and root planing on the amounts of interleukin-1 receptor antagonist and the mRNA expression of interleukin-1β in gingival crevicular fluid and gingival tissues. *Journal of Periodontal Research* **39**, 158–167.

Zanatta, G. M., Bittencourt, S., Nociti, F. H. Jr., Sallum, E. A., Sallum, A. W. & Casati, M. Z. (2006) Periodontal debridement with povidone-iodine in periodontal treatment short-term clinical and biochemical observations. *Journal of Periodontology* **77**, 498–505.

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**Clinical Relevance**

*Scientific rationale for the study*: The concern with bacterial recolonization and the knowledge that intentional removal of tooth structure is not a requirement for periodontal healing gave scientific support for the concept of periodontal debridement. The present study evaluates this approach as a therapeutic alternative to the treatment of severe chronic periodontitis.

*Principal findings*: Periodontal debridement led to similar clinical, microbiological and immunological outcomes when compared with standard scaling and root planing.

*Practical implications*: Periodontal debridement may be assumed as a viable approach to deal with severe chronic periodontitis. Long-term follow-up is required to confirm the good prognosis of this alternative approach.