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The recolonization hypothesis in a full-mouth or multiple-session treatment protocol: a blinded, randomized clinical trial

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Abstract

Aim: To test recolonization of periodontal lesions after full-mouth scaling and root planing (FM-SRP) or multiple session-SRP (MS-SRP) in a randomized clinical trial and whether FM-SRP and MS-SRP result in different clinical outcomes.

Materials and Methods: Thirty-nine subjects were randomly assigned to FM-SRP or MS-SRP groups. At baseline and after 3 months, probing pocket depth (PPD), plaque index (PlI) and bleeding on probing (BoP) were recorded. At baseline, immediately after treatment, after 1, 2, 7, 14 and 90 days, paper point samples from a single site from the maxillary right quadrant were collected for microbiological analysis of five putative pathogens by polymerase chain reaction.

Results: FM-SRP and MS-SRP resulted in significant reductions in PPD, BoP and PlI and the overall detection frequencies of the five species after 3 months without significant differences between treatments. Compared with MS-SRP, FM-SRP resulted in less recolonization of the five species, significantly for *Treponema denticola*, in the tested sites.

Conclusion: FM-SRP and MS-SRP result in overall clinically and microbiologically comparable outcomes where recolonization of periodontal lesions may be better prevented by FM-SRP.

Key words: bacteria; full-mouth-SRP; multiple session SRP; periodontitis; polymerase chain reaction; recolonization

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Periodontitis is an infectious, chronic and multifactorial inflammatory disease that affects the tooth-supporting tissues. Severe periodontitis can ultimately result in tooth loss. Bacteria associated with periodontal diseases are mainly Gram-negative species belonging to the phyla of the *Bacteroidetes*, *Fusobacteria* and *Spirochetes* (Socransky & Haffajee 2005). Besides the microbiological component, risk factors associated with the disease include behavioural factors such as stress and smoking as well as genetic traits (Kinane & Attström 2005, Tonetti & Claffey 2005). Non-surgical mechanical scaling and root planing (SRP) aims to reduce the total bacterial load and to remove periodontal pathogens from the subgingival area. Quadrant-wise SRP (Q-SRP) is usually performed in four or more subsequent sessions with weekly intervals. Together with oral hygiene instructions, SRP is the treatment of choice for an effective cause-related periodontal therapy (Cobb 1996). Improved clinical periodontal conditions have been associated with a reduction of the total supra- and subgingival bacterial load including *Spirochetes* and *Capnocytophaga* species (Slots et al. 1979), the percentage of sites positive for *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* (Darby et al. 2001) and a prolonged suppression of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *P. intermedia* (Shiloah & Patters 1996). Van der Velden et al. (1986) and van Winkelhoff et al. (1986) showed that periodontal

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pathogens could also be detected on the dorsum of the tongue and the oral mucosa. Together with the suggested translocation of bacteria from one site in the oral cavity to another, it was hypothesized that in between the subsequent sessions of Q-SRP, previously treated quadrants could be reinfected by bacteria from not yet treated quadrants (Quirynen et al. 1996, Greenstein & Lamster 1997, Quirynen et al. 2001).

Based on this reinfection hypothesis, the full-mouth disinfection (FMD) protocol was introduced by Quirynen et al. (1995) and included full-mouth SRP (FM-SRP) within 24 h. Furthermore, additional disinfection was sought by tongue brushing with chlorhexidine gel (1.0%), rinsing with chlorhexidine 0.2% twice daily and subgingival irrigation with 1% chlorhexidine gel.

The clinical outcome of the traditional Q-SRP and FM-SRP or FMD has been compared in several studies (Quirynen et al. 1995, Apatzidou & Kinane 2004, Koshy et al. 2005, Wennström et al. 2005, Jervøe-Storm et al. 2006, Quirynen et al. 2006, Swierkot et al. 2009). Recently, a meta-analysis by Eberhard et al. (2008) showed only differences in the weighted mean differences (WMD) between FMD and Q-SRP of 0.53 mm for PPD and 0.33 mm for clinical attachment level (CAL) in favour of FMD. When comparing FMD with FM-SRP the WMD for CAL amounted 0.74 mm in favour of FM-SRP. The included studies differed however in study design i.e. FMD or FM-SRP *versus* Q-SRP, included both smokers and non-smokers (Apatzidou & Kinane 2004, Wennström et al. 2005, Jervøe-Storm et al. 2006, Quirynen et al. 2006). Only the studies performed by Koshy et al. (2005), Wennström et al. (2005) and Jervøe-Storm et al. (2006) showed a low risk of bias based on randomization, allocation concealment, blinding and completeness of follow up (Eberhard et al. 2008). Finally, only the studies of Wennström et al. (2005) and Jervøe-Storm et al. (2006) were powered to detect predefined statistical differences in treatment outcomes. The presented literature shows that, within the limitations of the studies, FMD and FM-SRP and Q-SRP show minor differences in clinical treatment outcome. However, outcome of clinical trials do not prove nor deny the hypothesis of reinfection of treated periodontal pockets by bacteria. In the study of Quirynen et al. (1995), a significantly better reduction in the number of pathogens was observed in the FMD group 1 month after treatment. Other authors have not been able to show additional microbiological effects of FM-SRP over Q-SRP alone (Apatzidou et al. 2004b, Koshy et al. 2005, Jervøe-Storm et al. 2007) nor with the addition of povidone iodine (Koshy et al. 2005) or with subgingival irrigation with chlorhexidine gel, tongue brushing with chlorhexidine gel for 1 min and post-treatment rinsing daily with chlorhexidine (Swierkot et al. 2009) by polymerase chain reaction (PCR). In addition, RT-PCR analysis revealed no microbiological differences between the different treatment modalities after 1 day, and 1, 2, 4, 8, 12 or 24 weeks (Jervøe-Storm et al. 2007). However, microbiological samples from different pockets were pooled and/or taken months after treatment (Quirynen et al. 1995, Bollen et al. 1998, Apatzidou et al. 2004b, Koshy et al. 2005, Swierkot et al. 2009). The aim of the present study is, therefore, to test recolonization of periodontal lesions after FM-SRP or multiple session SRP (MS-SRP) in a randomized clinical trial and test whether FM-SRP and MS-SRP result in different clinical outcomes.

Materials and Methods

Experimental design and patient selection

The patients in this study were referred to a private clinic for periodontology in Groningen. After recording probing pocket depth (PPD), bleeding on probing (BoP), levels of supragingival plaque, presence of furcation lesions and medical history of the patient, an external examiner (V. Z.) selected 44 patients who where eligible and fit the inclusion criteria. Patients diagnosed with chronic periodontitis, aged 25–75 years and with >16 teeth and $>10\%$ of the sites with PPD$\geqslant$6 mm were candidates for inclusion. Patients were not admitted to the study if any of the following criteria were present: (1) smokers and former smokers who stopped <5 years ago, (2) use of local or systemic antibiotics 3 months before the study, (3) removable partial dentures, (4) pregnancy or lactation, (5) presence of systemic diseases requiring drug therapy and (6) periodontal treatment within the past 5 years. Patients participated in the study based on informed consent. The patients were stratified for the two trained and experienced ($\geqslant$8 years) oral hygienists who performed the treatment. The clinical protocol and the time-points for microbiological sampling are shown in Fig. 1.

The hygienists were instructed to start periodontal treatment in the maxillary right quadrant (test-quadrant), in order to obtain the highest level of operator blinding and the prevention of an operator bias. When the treatment was finished, a second independent person informed them whether they had to continue the treatment in the other quadrants (FM-SRP) or continue treatment in another session (MS-SRP), based on a computer-generated randomization table. After 3 months the patients were examined by a periodontist. All study personnel was blinded to treatment assignment for the duration of the study. The research protocol was approved by the Ethical Committee of the University Medical Center Groningen.

Treatment

FM-SRP

The patients that were assigned to the FM-SRP protocol received a full-mouth subgingival debridement with manual periodontal curettes (Hu-Friedy Manufacturing Co., Chicago, IL, USA) in a 3-h single session. Treatment was performed under local anaesthesia on patient's request. Patients received standard oral hygiene instructions including tooth brushing and inter-dental plaque control by inter-dental brushes. 1, 2, 7 and 14 days after treatment patients returned to the clinic for microbiological sampling. At days 7 and 14 the oral hygiene instructions were reinforced.

MS-SRP

The patients assigned to the MS-SRP protocol received subgingival debridement with manual periodontal curettes (Hu-Friedy Manufacturing Co.) in three sessions of 1 h at 1-week intervals according to the protocol of the clinic. Treatment was performed under local anaesthesia on patient's request. The first quadrant was always treated in the first session. The rest of the dentition was divided in two equal portions and treated in the two consecutive sessions. One and 2 days after the first treatment, patients returned to the clinic for microbiological sampling. At each treatment

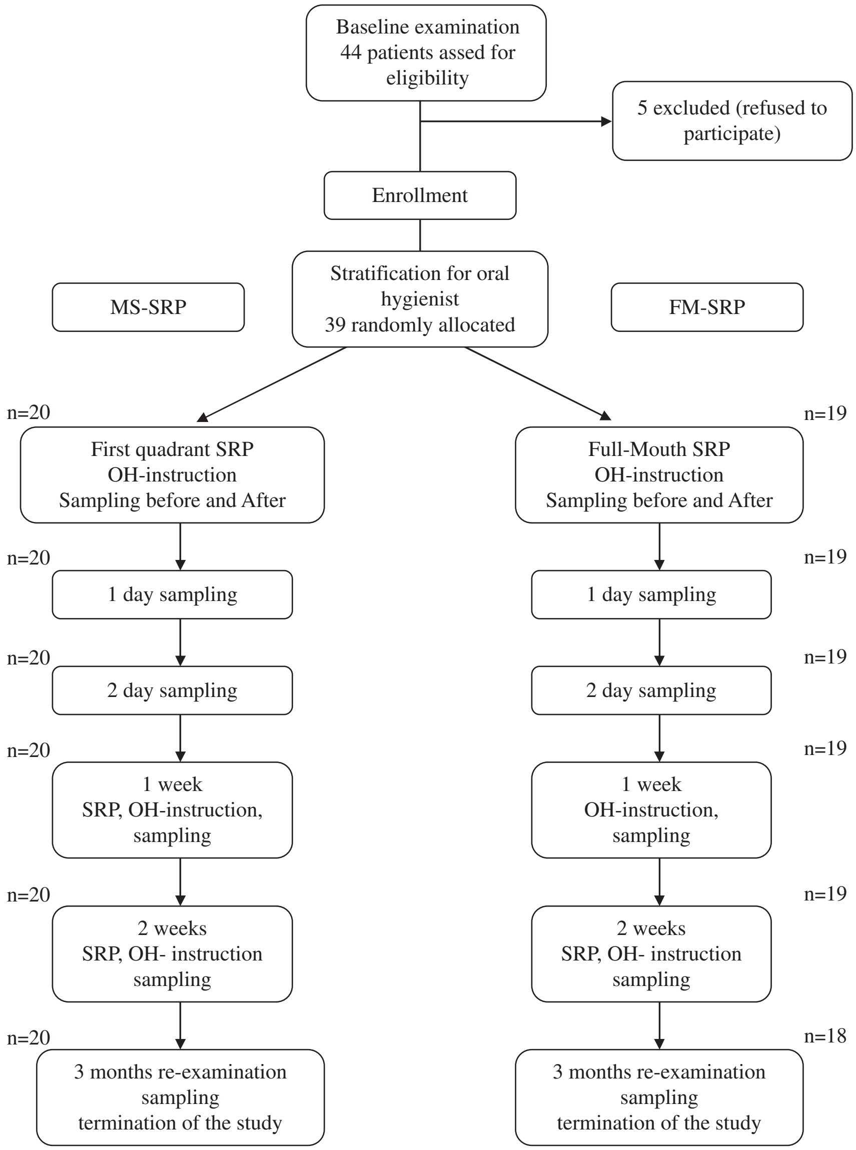

Fig. 1. Flowchart of the study outline. Of the 44 eligible patients, five refused to participate. One of the 39 enrolled patients decided to exit from the study before the re-examination session. This patient was enrolled to the full-mouth scaling and root planning (FM-SRP) group.

session, microbiological samples were collected and patients received standard oral hygiene instructions including tooth brushing and inter-dental plaque control by inter-dental brushes.

Clinical measurements

Before treatment and 3 months (3.5 months for the test-quadrant in the MS-SRP group) after completion of the treatment, clinical parameters were assessed by a blinded examiner. PPD to the nearest millimeter was assessed at six sites per tooth using a manual probe (PCP-UNC 12, Hu-Friedy Manufacturing Co.), and BoP (Van der Velden 1979) and plaque index (PlI) (Silness & Löe 1964) were recorded. According to the practice protocol, pockets measuring <3 mm were considered healthy and not recorded.

Microbiological sampling

In each quadrant, a single pocket with PPD$\geqslant$6 mm on a single rooted tooth was selected by the external examiner. Microbiological samples from this specific tooth in the test-quadrant were collected at seven time-points in the test-quadrant: before treatment, immediately after SRP, 1 day, 2 days, 1 week, 2 weeks and 3 months after treatment. The other quadrants were sampled before treatment, immediately after treatment and after 3 months. After removal of supragingival plaque and the isolation of the site with cotton rolls, sampling was performed with a single sterile paper point (ROEKO®, size M, Coltene/Whaledent GmbH, Langenau, Germany), which was left in place for 20 s. Samples were collected in coded screw-cap tubes and transported to the laboratory and stored at -20°C until further processing.

DNA extraction

DNA was extracted according to the extraction protocol of Zijnge et al. (2006) with minor modifications. 200 μl of demineralized H_20 and four glass beads were added to the tubes with the paper points. After homogenizing thoroughly for 5 s using a vortex, three cycles of freeze–thawing at -80°C for 15 min and 5 min at 80°C were performed. Subsequently, the samples were incubated for 1 h at 37°C with 10 μl lysozyme (40 mg/ml), followed by an incubation for 1 h at 58°C with 100 μl lysis buffer (10% SDS, 0.2 mg/ml proteinase K). Proteinase K was inactivated by incubation at 80°C for 10 min. For DNA isolation, 200 μl phenol and 200 μl chloroform/iso-amylalcohol (24:1 v/v) were added to the samples. The samples were centrifuged for 5 min at 14,000 *g*. A second phenol/chloroform/iso-amylalcohol extraction was performed on the aqueous phase and centrifugation, DNA was precipitated from the aqueous phase with 1/10 v/v 3 M sodium acetate (pH 5.2) and 2.5 v/v 96% ethanol at –20°C overnight. After centrifugation for 15 min at 14,000 *g*, the supernatant was discarded and the pellet washed twice with 100 μl 70% alcohol. After centrifugation for 15 min at 14,000 *g*, the supernatant was removed. The pellet was dissolved in 50 μl sterile TE buffer and stored at –20°C.

Species-specific PCR

PCR for the detection of *P. gingivalis* (Pg), *A. actinomycetemcomitans* (Aa), *T. forsythia* (Tf) and *T. denticola* (Td) was performed according to Zijnge et al. (2006). For the detection of *Fusobacterium nucleatum* (Fn) the primers Fn607-GCGCGTCTAGGTGGTTATGTAA and Fn1060-CTGTCTTTAGGTTTCCCCGAAG were developed using the ARB software package (Ludwig et al. 1998). These primers were optimized and tested for sensitivity and specificity with strain *F. nucleatum* ATCC 25586 and against a panel of reference strains with the PCR protocol by Zijnge et al. (2006) for species-specific PCR. For the PCR reactions, the limit of detection was 50 cells.

Statistical analysis

The clinical hypothesis to test is whether FM-SRP and MS-SRP results in different reductions in PPD. The primary response variable is therefore PPD. According to Wennström et al. (2005), 20 patients in each treatment group were required based on an expected mean difference in PPD between groups of 0.5 mm and a common standard deviation of 0.6 mm. During the course of the study, a meta-analysis by Eberhard et al. (2008) precised the expected mean difference in PPD between the two treatment groups to 0.53 mm. With a common standard deviation of 0.6 mm, the α-error predefined to 0.05 and the β-error to 0.2, a power analysis for a two-tailed t-test for independent means revealed that in each group 22 patients were required. In all tests, the patient was set as the experimental unit. Change in BoP and PlI was defined as the percentage of sites that were positive at baseline and negative for respectively bleeding and visible plaque after 3 months. The percentage of healthy pockets is defined as the percentage of the pockets for which PPD $\geqslant$ 5 mm at baseline were reduced to PPD $\leqslant$ 3 mm after 3 months.

Within group changes in PPD between baseline and after 3 months were tested with a paired two-tailed t-test. Differences in PPD between FM-SRP and MS-SRP were tested with a two-tailed t-test for independent means. Pockets measuring $<$3 mm after 3 months were set to 3 mm to be able to calculate an average PPD.

Within group differences in BoP and PlI between baseline and after 3 months were tested with the non-parametric Wilcoxon test while differences between FM-SRP and MS-SRP were tested with the non-parametric Mann–Whitney test.

Within group changes for the detection of the five species between baseline and after 3 months were tested by the non-parametric McNemar test while differences between FM-SRP and MS-SRP were tested with the non-parametric Mann–Whitney test.

Timeline bacterial results of the test-quadrant pocket were categorized into predefined categories. ''Success'' was defined as when a pocket was positive for a species at baseline and continuously became negative for that species after treatment until the end of the study. ''Failure'' was defined as when a pocket was positive for that a species at every time-point. ''Recolonization'' was defined as when a positive pocket that became negative for a species and showed positive thereafter in the course of the study. ''Neutral'' was defined as pockets that were negative for a species and remained negative during the study. Only pockets with baseline values that could possibly result in ''Success'', ''Failure'' or ''Recolonization'' were considered for statistical analysis. Differences in category distribution between FM-SRP and MS-SRP were tested for by the χ^2-test except when expected counts were $<$5 where the Fisher's exact test was used. The level of significance was set to $p<0.05$.

The SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA) was used for data handling and statistical testing.

Results

Study descriptives

Between September 2007 and December 2008, 44 patients were recruited that fulfilled the inclusion criteria. Of the patients who attended the baseline examination, five refrained to participate and one person, originally assigned to the FM-SRP group, dropped out 10 weeks after treatment for financial reasons. In total, 38 patients completed the follow-up of the study. For statistical analysis, only the data of the included patients that completed follow-up of the study were used. This resulted in a power of 0.75, which is the probability that this study rejected a false hypothesis. Demographic characteristics are summarized in Table 1. There were no significant differences between FM-SRP and MS-SRP groups for baseline values of PPD, BoP and PlI in the whole mouth. There was only a significant difference in PPD of deep pockets in the test-quadrant at baseline (Table 2). There were no reports of adverse events or severe side effects of both treatments.

Clinical effects of treatment

The results of the test-quadrant and whole-mouth analyses showed no significant clinical differences within each treatment group (data not shown), and whole-mouth results were used for hypothesis testing. In general, both FM-SRP and MS-SRP resulted in significant reductions in PPD compared with baseline values. There were no significant differences in PPD reduction between FM-SRP and MS-SRP (Table 3). This result was confirmed by the absence of a significant difference between FM-SRP and MS-SRP with respect to the percentage of pockets initially measuring $\geqslant$5 mm and which were reduced to $\leqslant$3 mm and considered healthy or remained $\geqslant$5 mm after 3 months. FM-SRP and MS-SRP showed significant improvements after 3 months in BoP and PlI, without significant differences between FM-SRP and MS-SRP (Table 3).

Table 1. Demographic and baseline characteristics of the patients

Patient	FM-SRP	MS-SRP
No. of subjects	18	20
Age (years)	47 ± 9	54 ± 10.2
No. of male:female	10:8	12:8
No. of teeth	27.5 ± 1.5	27.2 ± 1.9

FM-SRP, full-mouth scaling and root planning; MS-SRP, multiple session-SRP.

Microbiological effects of treatment

Microbiological observations in the test-quadrant showed that FM-SRP and MS-SRP resulted in significant reductions in the number of pockets positive for *T. denticola* and *T. forsythia* compared with baseline. No significant reductions in *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* were observed after treatment. When samples from all four quadrants were analysed, there was also a significant reduction in the number of pockets positive for *P. gingivalis* at the end of the study. Between the two treatment protocols there were no significant differences in the reduction of the number of pockets positive for *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *F. nucleatum* and *T. forsythia* after 3 months (Table 4).

Changes in the frequency of detection of *T. denticola*, *F. nucleatum* and *T. forsythia* in the pockets of the test-quadrant are represented on a timeline in Fig. 2. Mechanical treatment itself had a limited effect on the elimination of *T. denticola*, *F. nucleatum* or

T. forsythia and was comparable between treatment modalities. In the course of the first week after treatment a continuing reduction in the percentage of positive pockets could be observed without additional mechanical intervention. This reduction was more pronounced in the FM-SRP treatment group. After the second and third session of SRP, the percentage of pockets positive in the MS-SRP continued to reduce. At the end of the observation period, only minor differences were observed between both treatment groups.

The frequencies of detection provide an average view instead of showing the effect of SRP on a specific species in a specified pocket. Therefore, the microbiological results of the pockets in the test-quadrant were categorized into four groups defined as ''success'', ''failure'', ''recolonization'' and ''neutral'' (Table 4). Although all five species responded more favourably to FM-SRP, only a trend in ''success'' between FM-SRP and MS-SRP was observed only for *T. forsythia* ($p = 0.095$). For *F. nucleatum*, the category ''failure'' was significantly higher ($p = 0.02$) in the MS-SRP group compared with the FM-SRP group. A significant difference in ''recolonization'' between FM-SRP and MS-SRP was found for *T. denticola* ($p = 0.043$) and a trend ($p = 0.061$) for *T. forsythia*.

Table 2. Probing pocket depth (PPD) results in the test-quadrant for moderate (4–6 mm) and deep pockets ($\geqslant$7 mm), the percentage of healthy pockets obtained (PPD $\leqslant$ 3 mm) and pockets that remain deep (PPD $\geqslant$ 5 mm) after 3 months and bleeding on probing (BoP) and plaque index (PlI) results (mean ± SD)

Test quadrant	Baseline		3 months		Change baseline-3-months		Pockets initial $\geqslant$5 mm		Baseline		Relative change baseline-3-months	
	moderate 4–6 mm	deep $\geqslant$7 mm	moderate 4–6 mm	deep $\geqslant$7 mm	moderate 4–6 mm	deep $\geqslant$7 mm	(healthy) PPD $\leqslant$3 mm	PPD $\geqslant$5 mm	BoP (%)	PlI (%)	BoP (%)	PlI (%)
FM-SRP	4.90 ± 0.23	7.77 ± 0.62	3.80 ± 0.55	6.25 ± 1.32	1.18 ± 0.38*	1.59 ± 0.84*	37.1 ± 17.0	51.8 ± 21.0	70.3 ± 23.0	70.6 ± 22.3	42.9 ± 26.7*	71.8 ± 19.1*
MS-SRP	4.91 ± 0.31	7.31† ± 0.38	3.71 ± 0.49	5.78 ± 1.04	1.27 ± 0.32*	1.69 ± 0.76*	33.0 ± 21.0	46.0 ± 22.0	65.5 ± 22.7	73.4 ± 22.1	43.5 ± 17.1*	59.9 ± 29.1*

*Significant change from baseline ($p < 0.05$).
†Significant difference between FM-SRP and MS-SRP ($p < 0.05$).
FM-SRP, full-mouth scaling and root planning; MS-SRP, multiple session-SRP.

Table 3. Probing pocket depth (PPD) results in the whole mouth for moderate (4–6 mm) and deep pockets ($\geqslant$7 mm), the percentage of healthy pockets obtained (PPD $\leqslant$ 3 mm) and pockets that remain deep (PPD $\geqslant$ 5 mm) after 3 months and bleeding on probing (BoP) and plaque index (PlI) results (mean ± SD)

Whole mouth	Baseline		3 months		Change baseline-3-months		Pockets initial $\geqslant$5 mm		Baseline		Relative change baseline- 3-months	
	moderate 4–6 mm	deep $\geqslant$7 mm	moderate 4–6 mm	deep $\geqslant$7 mm	moderate 4–6 mm	deep $\geqslant$7 mm	(healthy) PPD $\leqslant$3 mm	PPD $\geqslant$5 mm	BoP (%)	PlI (%)	BoP (%)	PlI (%)
FM-SRP	4.82 ± 0.18	8.81 ± 0.52	3.70 ± 0.46	7.07 ± 1.31	1.12 ± 0.39*	1.74 ± 1.15*	31.8 ± 15.0	50.5 ± 15.6	64.8 ± 25.1	74.2 ± 19.5	46.8 ± 22.1*	61.2 ± 17.2*
MS-SRP	4.83 ± 0.27	8.61 ± 0.25	3.64 ± 0.36	6.54 ± 0.71	1.18 ± 0.29*	2.07 ± 0.78*	32.5 ± 15.0	48.2 ± 18.0	66.9 ± 18.2	79.7 ± 18.0	49.4 ± 15.7*	64.1 ± 22.3*

*Significant change from baseline ($p < 0.05$).
FM-SRP, full-mouth scaling and root planning; MS-SRP, multiple session-SRP.

Discussion

The hypothesis formulated by Quirynen et al. (1995) was that reinfection of a disinfected area might challenge periodontal treatment outcome. Periodontitis is considered a multifactorial disease in which a highly diverse microbial population is considered causative (Page & Kornman 1997). It has however not been possible to identify a single bacterial species that fulfil the postulates of Koch for true pathogens. Moreover, microbiological detection methods have an inherent detection limit and therefore cannot be used to show the true absence of a species. However, it might not be important to detect the true absence of periodontal pathogens as they can also be found in healthy individuals (Ximénez-Fyvie et al. 2000). Furthermore, plaque is a biofilm in which multiple species cooperate. When plaque matures, the number of gram negative species increase. In some

Table 4. Treatment results in the test-quadrant (18 or 20 pockets) and between brackets all 4 quadrants (72 or 80 pockets) for the presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Fusobacterium nucleatum* and *Tannerella forsythia* in the FM-SRP ($N = 18$) and MS-SRP ($N = 20$) group after 3 months and the distribution of the species in the tested pockets over the different categories

	Number of pockets positive for a species			Category distribution (# pockets)			
	before	after	decrease	success	failure	recolonization	neutral
FM-SRP							
A. actinomycetemcomitans	2 (6)	1 (6)	1 (0)	1	0	1	16
P. gingivalis	9 (36)	4 (22)	5 (14*)	5	2	3	8
T. denticola	16 (62)	3 (20)	13* (42*)	13	1	2†	2
F. nucleatum	18 (71)	16 (69)	2 (2)	2	9†	7	0
T. forsythia	17 (65)	7 (37)	10* (28*)	11	4	3	0
MS-SRP							
A. actinomycetemcomitans	2 (9)	3 (6)	− 1 (3)	1	0	4	15
P. gingivalis	10 (40)	6 (25)	4 (15*)	4	3	5	8
T. denticola	14 (63)	7 (22)	7* (41*)	8	1	8†	3
F. nucleatum	20 (80)	20 (78)	0 (2)	0	17†	3	0
T. forsythia	19 (76)	12 (44)	7* (32*)	7	4	9	0

*Significant decrease from baseline ($p < 0.05$).
†Significant difference between FM-SRP and MS-SRP ($p < 0.05$).
FM-SRP, full-mouth scaling and root planning; MS-SRP, multiple session-SRP.

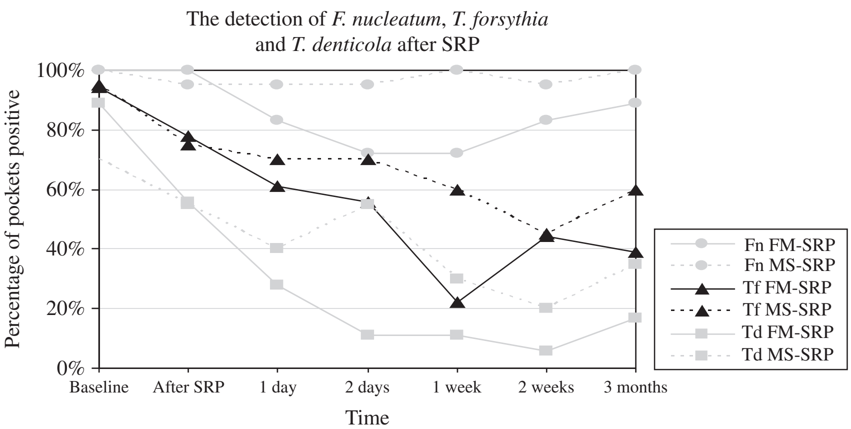

Fig. 2. The percentage of tested pockets in the test-quadrant that was positive for *Fusobacterium nucleatum*, *Tannerella forsythia* and *Treponema denticola* at baseline and at different time-points after full-mouth scaling and root planning (FM-SRP) or multiple session-SRP (MS-SRP).

patients, this may lead to the development of periodontitis, in others not. Hence, even in the presence of so-called periodontal pathogens, a susceptible host is needed for periodontitis to develop, as presented by the pathogenesis model in Page & Kornman (1997). Because the term ''infection'' also implies a host response, we consider the term recolonization more appropriate to study bacterial (re) appearance.

The aim of the present study was to test the recolonization of periodontal lesions after FM-SRP or MS-SRP in a randomized clinical trial and test whether FM-SRP and MS-SRP result in different clinical outcomes. The setting of this study was a private clinic for periodontology requiring compromises on trial design. The protocol of the clinic demanded for example a three session SRP protocol and did not include the registration of pockets < 3 mm and CAL. We believed that a three session SRP protocol was still suitable for testing the recolonization hypothesis because in this setting there were remaining quadrants that could serve as a reservoir for periodontal pathogens. CALs are prone to measurement errors, especially in inflamed periodontal tissues (Van der Velden & Jansen 1980). PPD was therefore regarded as the appropriate measure for short-term periodontal treatment outcome. This study was designed as a randomized clinical trial according to the guidelines set by the Consort group CONSORT (2001) for the blinding of the oral hygienists, randomization concealment, completeness of follow up and an a priori power analysis to determine sample size. Blinding of the oral hygienists who performed the SRP was sought by designing the upper right quadrant as the test-quadrant. Moreover, in the present study, patients were stratified for the oral hygienists, thereby reducing eventual intra-operator differences that might have biased the clinical outcomes. Analysis of the test-quadrant results and the whole-mouth clinical data revealed no statistical differences and whole-mouth data were therefore used for hypothesis testing. With the inclusion of 18 (FM-SRP) and 20 (MS-SRP) instead of the 22 required subjects in each group, this study reached a power of 0.75 of drawing the correct conclusion when the null-hypothesis that FM-SRP and MS-SRP result in equal reductions in PPD would be rejected.

For microbiological measurements a single pocket in the test-quadrant was selected to monitor recolonization, because from a microbiological point of view the pocket is the ecological determinant. However, sampling multiple pockets from the test-quadrant would have increased the strength of the analysis. This was however beyond our logistical capabilities.

In general, both FM-SRP and MS-SRP resulted in significant reductions in PPD compared with baseline values. The reductions in PPD in the MS-SRP group were comparable to meta-analysis results from the studies of Badersten et al. (1981), Badersten et al. (1984) and Cobb 1996. FM-SRP resulted in slightly less reductions in PPD with 1.12 mm in initial moderate and 1.74 mm and initial deep pockets. That FM-SRP results in lesser, however not significantly, differences in the reductions in PPD has also been observed by Apatzidou et al. (2004b), Koshy et al. (2005) and Jervøe-Storm et al. (2006), but not by others (Quirynen et al. 1995, Wennström et al. 2005, Swierkot et al. 2009).

After 3 months, there were no significant differences between FM-SRP and MS-SRP in the overall reduction of sites positive for *P. gingivalis*, *T. denticola* and *T. forsythia*. Considering the sampled pockets in the test-quadrant, however, FM-SRP was more successful in eliminating the five species tested, although not significantly. There are two possible explanations for this observation. First, recolonization occurred more often in the MS-SRP group as compared with the FM-SRP group and was significant for *T. denticola* (Table 4). This may be the result of a lower reduction in PlI in the test-quadrant of the MS-group. In the presence of high post-treatment plaque levels, periodontal pathogens may reach pre-treatment levels in 3 weeks (Rhemrev et al. 2006). The second explanation might be that although immediately after the initial session of SRP in the FM-SRP and MS-SRP group, only a limited reduction in the sites positive for *T. denticola, F. nucleatum* and *T. forsythia* was detected; an ongoing reduction in positive sites could be observed up to 1 and 2 weeks, without additional SRP of this quadrant. For FM-SRP this was more pronounced and is possible due to an immunological effect on the bacteria in the biofilm. We speculate that a single session FM-SRP provokes a quantitatively more pronounced acute immune response as compared with MS-SRP. This quantitative difference in the immune response may explain the stronger reduction in the detection frequencies of the pathogens by FM-SRP found in this study. Interestingly, the subsequent sessions of SRP in the MS-SRP group resulted in an ongoing reduction in the detection frequencies in the test-quadrant without additional SRP in that quadrant, resulting in the absence of significant differences between the two groups after 3 months. This resembles the Schwartzman reaction or the vaccine effect (Page 2000, Quirynen et al. 2000). Apatzidou & Kinane (2004a), Wang et al. (2006), on the other hand, showed that both treatment modalities did not result in increased levels of IgG to *P. gingivalis*, *T. denticola*, *P. intermedia*, *T. forsythia* or *A. actinomycetemcomitans* during the active phase of treatment but with increased avidity.

FM-SRP shows significantly lesser recolonization of *T. denticola* in the sampled pocket of the test-quadrant but did not result in a significant difference in the overall detection frequency of the five pathogens after 3 months as compared with MS-SRP. In contrast, MS-SRP appears to result in slightly better, but not significant, clinical treatment outcomes as compared with FM-SRP. Reflecting on this, the periodontitis pathogenesis model is helpful (Page & Kornman 1997). From this model, it becomes clear that the clinical features of periodontitis are the result of the interaction of the bacterial component, host immune responses and periodontal tissue metabolism. The mere presence or absence of a single species as a determinant for clinical success or failure might therefore be regarded as a simplification of the complexity of the disease. Further studies on this topic are strongly recommended to include short time and site-specific immunological parameters of both the innate and humoural immune response in addition to microbiological parameters.

In conclusion, the present study shows that FM-SRP and MS-SRP do not result in different clinical outcomes for PPD, BoP and PlI and the overall detection frequencies of five periodontal pathogens after 3 months. Confirmatory to the recolonization hypothesis, FM-SRP shows less recolonization as compared with MS-SRP. This argument however should be used with care to support a treatment modality as both result in equally good and acceptable clinical outcomes. Both treatment modalities can be considered for initial non-surgical periodontal treatment according to patients' needs and preferences, operator skills, practice settings and cost-effectiveness (Lang et al. 2008, Sanz & Teughels 2008) and will result in anticipated clinical outcomes.

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Clinical Relevance

Scientific rationale for the study: The clinical outcome of SRP of subgingival pockets in subsequent sessions might be challenged by recolonization of already treated sites from not yet treated sites.

Principal findings: FM-SRP and MS-SRP result in comparable, significant overall clinical and microbiological improvements. FM-SRP prevents recolonization.

Practical implications: Considering the good clinical outcomes of both treatment modalities, the argument of recolonization is of limited value in choosing a preferred treatment option.