Hyaluronic Acid as an Adjunct After Scaling and Root Planing – A Prospective Randomized Clinical Trial

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Aim: The study was designed to determine the effect on clinical variables, subgingival bacteria and local immune response brought about by additional application of hyaluronan-containing gels in early wound healing after scaling and root planing (SRP).

Material and Methods: In this randomised clinical study, data from 34 individuals with chronic periodontitis was evaluated after full-mouth SRP. In the test group (n = 17), hyaluronan gels in two molecular weights were additionally applied during the first two weeks after SRP. The control group (n = 17) was treated with SRP only. Probing depth (PD) and attachment level (AL) were recorded at baseline and after 3 and 6 months, and subgingival plaque and sulcus fluid samples were taken for microbiological and biochemical analysis.

Results: In both groups, PD and AL were significantly reduced ($p < 0.001$). The changes in PD and the reduction of the numbers of pockets with PD ≥ 5mm were significantly higher in the test group after 3 ($p = 0.014$; $p = 0.021$) and 6 months ($p = 0.046$; $p = 0.045$). Six months after SRP, the counts of *Treponema denticola* were significantly reduced in both groups (both $p = 0.043$), those of *Campylobacter rectus* in the test group only ($p = 0.028$). *Prevotella intermedia* and *Porphyromonas gingivalis* increased in the control group.

Conclusions: The adjunctive application of hyaluronan may have positive effects on probing depth reduction and may prevent recolonization by periodontopathogens.

KEY WORDS:

chronic periodontitis; root planing; hyaluronic acid; microbiology; leucocyte elastase

Scaling and root planing (SRP) are effective methods in the treatment of periodontal diseases.[1] Findings from a systematic review have shown that subgingival mechanical debridement results in a mean attachment gain of up to 1.58 mm in pockets with an initial depth ≥ 7 mm.[2] Different local antimicrobial and anti-inflammatory adjuncts have been shown to improve the outcome of SRP. Antibiotics, e.g. tetracyclines,[3, 4] metronidazole[5] were applied to the periodontal pockets; in general, results for SRP combined with topical antibiotics were not or were only marginally better than for SRP without antibiotics.[5, 6]

Another possible approach is the application of hyaluronan (or hyaluronic acid, HA). HA is a non-sulfated glycosaminoglycan and a major component in the extracellular matrix.[7] In human periodontal ligament cells, fibroblast-growth factor-2 regulates the production of HA.[8] Although high levels of glycosaminoglycans are detectable in the gingival crevicular fluid of periodontitis patients, the amount is reduced after periodontal therapy.[9] In addition, glycosaminoglycans with a high molecular weight are found in periodontally healthy individuals: the molecular size is lower in periodontitis patients, suggesting degradation of the

molecules.[10] Lower molecular-mass forms of HA but not the native forms induce inflammation reduction by means of signaling through toll-like receptors (TLR) 2 and 4.[7]

In in-vitro and in animal studies, the application of hyaluronan showed positive effects on fibroblasts, bone regeneration and wound healing.[11-13] Hyaluronan acts as an anti-inflammatory.[14, 15] HA is currently under discussion as a treatment option in osteoarthritis,[16] urinary incontinence in women[17], and is already in use as a soft tissue filler.[18] In dentistry, HA has showed a positive effect on the reduction of plaque and on the sulcus bleeding index of patients with plaque-induced gingivitis.[19, 20] Only in a very few studies has HA been applied as an adjunct to scaling and root planing in non-surgical treatment of periodontitis. Johannsen et al.[21] reported significant reductions of BOP and PD after the adjunctive use of subgingivally applied 0.8% HA gel immediately post SRP and 1 week afterwards. However, in another study applying 0.2% HA gel weekly for 6 weeks after SRP in chronic periodontitis patients, no influence of HA on clinical variables or on periodontopathogens was found after 6 and 12 weeks.[22]

The objective of the present study was to determine the effect on clinical variables, subgingival periodontopathogenic bacteria and local immune response brought about by the additional use of a 0.8% HA gel during SRP and of a 0.2% HA gel used twice daily for two weeks after SRP.

MATERIAL AND METHODS

Patients

Following approval of the study by the Ethics Commission (#121-2006) of the University of Leipzig Medical Faculty, forty-two randomly selected volunteers (24 female and 18 male) gave written and informed consent to their participation in the randomised non-blinded clinical study in the Department of Periodontology at the University of Leipzig in 2007 and 2008.

Only individuals with moderate or severe chronic periodontitis[23] with at least 5 sites with probing depths (PD) ≥ 5 mm and a minimum of 20 teeth were included in the study. The interproximal plaque index (API)[24] was required to be below 30% after two initial prophylaxis and instruction sessions. Individuals were excluded if they had taken antibiotics in the six months prior to the study or if they had received periodontal treatment during the previous year. Pregnancy, nursing, smoking, chronic diseases such as diabetes mellitus or rheumatoid arthritis and allergy to ingredients in the drug were also criteria for exclusion.

All treatment was performed by the same dentist (M.H.). To avoid bias, plaque sampling, GCF and assessment of the clinical data were performed by another investigator blinded to the treatment (H.J.). Treatment assignment was performed by an assistant in accordance with a computer-generated randomization table. The 42 patients were allocated into a test group consisting of 21 and a control group of 21 participants.

The clinical variables PD, attachment level (AL) and bleeding on probing (BOP) of all teeth were determined in a 4-point measurement per tooth (mesiobuccal, buccal, distobuccal and midoral) with a manual periodontal probe** at three appointments: before SRP (baseline, t0), after 3 months (t1) and 6 months (t2). The API was also recorded. The interproximal area was considered as one site for the purposes of recording the API. At the same time, samples of the subgingival biofilm and gingival crevicular fluid (GCF) were taken from the deepest site in both the premolar and the molar regions.

Four samples were taken per volunteer. First, paper strips†† were placed at the entrance of the periodontal pocket for 20 s. This method ensures that the subgingival biofilm in the

pocket is not destroyed. Following this, endodontic paper points[‡‡] were inserted into the pocket until resistance was felt and were left in place for 30 s. The strips and points were stored as a pooled sample at -20°C immediately after sampling. The frozen samples were transferred within two weeks to the laboratory where the plaque samples were again stored at -20°C, and the GCF samples at -80°C for a maximum of nine months before analysis.

Therapy and Follow-Up Treatment

After professional tooth cleaning and motivation and instruction of the patients regarding oral hygiene, the interproximal plaque index was ≤ 30 %. Under local anaesthesia with articaine hydrochloride/epinephrine hydrochloride,[§§] the participants received full-mouth scaling and root planing in two sessions carried out within 24 hours using hand and ultrasonic instruments. All patients used a chlorhexidine digluconate mouthwash[***] for one minute twice daily during the first seven days after SRP and carefully performed normal oral hygiene with toothbrush and interdental brushes.

Immediately after the SRP, a 0.8% HA (1,800 kDa)-containing gel[†††] was introduced into all periodontal pockets in the test group (n = 21) by the periodontist (M.H.). In addition, the patients applied a 0.2% HA (1,000 kDa)-containing gel[‡‡‡] onto the gingival margin twice daily over the following 14 days. They were asked to cover the buccal and oral gingiva with the gel to excess and to direct the excess into the interproximal area. It is legally stipulated that the 0.8% HA gel may be applied only by the dentist, while the 0.2% gel may be used by the patient at home.

The control group (n = 21) was treated with SRP only; no placebo was used.

Biochemical and Microbiological Analysis

Immediately before analysis, GCF samples were eluted overnight into 500 µl phosphate buffered saline at 4°C. Neutrophil elastase (NE) activity was determined with a microplate assay using the chromogenic substrate N-methoxysuccinyl-Ala-Ala-pro-Val-pNa[#]. The substrate was dissolved in dimethylsulphoxide (DMSO) to 10 mM, and the working solution was 1 mM after dilution with 0.05 M Tris, pH 7.5. In short, 10 ml of the substrate working solution was added to each 90 ml of the eluate from the specimen. Absorption at 405 nm was measured immediately and also after incubation at 37°C for 30 min in a microplate reader. NE activity in GCF is measured as an increase in absorption. The assay used for the determination of the activity of myeloperoxidase has been described by de Mendez *et al.* [25] The substrate includes Triton-X-100, *o*-dianisidine and H_2O_2 in sodium citrate buffer. The absorption at 450 nm was measured immediately in a microplate reader. After incubation for 30 min at 37°C, the measurement was repeated. These measurements were also performed including sodium azide as a myeloperoxidase inhibitor.[26] The readings of substrate and sample were subtracted from the values including additional inhibitor.

DNA was extracted from the plaque samples using a kit[§§§]. The subsequent quantitative detection of selected periodontodontopathogenic bacterial species (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Tannerella forsythia, Treponema. denticola, Prevotella intermedia* and *Campylobacter rectus*), was performed by means of real-time polymerase chain reaction (PCR), as recently described by Eick.[27]

Data Analysis

The null hypothesis of our study was that there are no statistically significant differences in the clinical parameters PD, AL and BOP between the test and control groups. The primary outcome variable was the change in the mean PD. Secondary outcome variables were changes in the number of sites with PD ≥ 5 mm, occurrence of BOP, mean AL, activity levels of

neutrophil enzymes and the counts of selected pathogenic bacteria associated with periodontitis.

Statistical analysis of the clinical and laboratory data was undertaken using software****. Unit of analysis in all statistical tests was the individual. Probing depth (PD) was set as the primary outcome and used to determine sample size. A mean difference of 1 mm in observed PD with a standard deviation of 1 mm between two groups or two examination dates would require ≥ 16 patients per group in order to detect a significant difference ($p \leq 0.05$) with a test power of 80%. The results are presented as PD and AL for all sites in a four-point measurement. The PCR data collected was analyzed as total counts of selected pathogenic bacteria, and also qualitatively. For both intra- and inter-group testing, non-parametric tests (Wilcoxon and U-test respectively) were used and X^2-test was performed for qualitative analysis of the presence of periodontopathogens. A level of $\alpha \leq 0.05$ was considered significant.

RESULTS

Figure 1 gives the study flow adapted to Moher et al.[28] The clinical results of the final 17 test and 17 control patients at baseline are given in Table 1. The mean PD was 4.2 ± 0.4 mm in the test group and 4.1 mm ± 0.4 mm in the control group. The mean AL was 5.5 mm ± 0.9 mm in the test group and 5.7 mm ± 0.6 mm in the control group. The corresponding BOPs were 16.3% ± 8.7% and 18.8% ± 11.1%. There were no significant differences between the groups at baseline. No adverse effects of HA were observed during the study in the years 2007 and 2008.

Changes in clinical data observed during the study are presented in Table 2. Significant improvements were detected for PD and AL in the test and control groups. Analysis of differences between the two groups revealed significant PD improvement in the test group compared to the control group after three and after six months (p = 0.014 and p = 0.046). Similarly, the numbers of sites with a PD ≥ 5 mm were reduced more in the test group than in the control group (p = 0.021 and p = 0.045). No differences were observed between the groups in changes in AL, BOP and API.

NE activity increased in correspondence with increased occurrence of BOP in the six months after treatment. When compared to baseline, NE activity was significantly increased after three and six months in both treatment groups (test group: p = 0.002, p = 0.019; control group: p = 0.003, p = 0.028). MPO activities did not change significantly. No significant differences were detected between the groups for NE and MPO activities (Figure 2).

Six periodontopathogenic bacteria were examined. At baseline, about 40% of the patients tested positive for *A. actinomycetemcomitans* and 60% for *P. gingivalis*. Differences between the test and control groups were found only six months after SRP. At the six-month-appointment, the prevalence both of *A. actinomycetemcomitans* (p = 0.027) and *C. rectus* (p = 0.008) was higher in the control group than in the test group (Table 3). Quantitative analysis found a decrease of the *T. denticola* counts in the test group six months after SRP over baseline (p = 0.043), and in the control group after three months in comparison with baseline (p = 0.043). Changes in *T. forsythia* were without significance in both groups. Furthermore, *C. rectus* counts decreased in the test group six months after SRP in comparison with baseline (p = 0.028). In contrast, at the 6-month examination in the control group, the counts of *P. intermedia* were significantly higher compared with baseline (p = 0.043) and those of *P. gingivalis* were increased compared with the appointment 3 months after SRP (p = 0.016; Figure 3).

DISCUSSION

This study analyzed the effect of an additional application of HA gels during SRP and the early wound-healing period up to 14 days. Clinical variables, inflammatory markers and selected periodontopathogens were examined after three and six months. In contrast to published studies[21, 22, 29], a combination of two gels was applied. A 0.8% HA (1,800 kDa)-containing gel was introduced into all periodontal pockets during SRP and followed by the application of a 0.2% HA (1,000 kDa)-containing gel onto the gingiva twice daily for 14 days after SRP. The control group did not receive a placebo gel as this was unavailable. This may constitute a weakness in the study.

In both treatment groups, a reduction of PD and AL was observed. The improvements were in the range of other post-SRP studies.[2, 30] The improvement in PD was more noticeable in the test group in comparison to the control group, suggesting a positive effect of hyaluronan on wound healing. The difference in full-mouth PD between the groups was 0.29 mm after 6 months, which is slightly less than results reported for systemically applied adjunctive amoxicillin/metronidazole in chronic periodontitis patients.[31] Our result is inconsistent with studies by Engström et al.[29] and Xu et al.[22], who did not find any difference in PD between HA test and control groups after treatment. It may be speculated that the usage of 1,800 kDa hyaluronan is of great importance for healing and clinical outcomes, especially in the first days and weeks after treatment, as considered in our study. The study by Johannsen et al.[21], who applied also a 0.8% HA gel subgingivally, demonstrated a higher, significant improvement of BOP in the HA group in comparison with SRP only. An improvement in AL and gingival recession was reported very recently in 14 patients with intrabony defects treated with periodontal surgery and HA.[32]

In our study, HA seemed to have an antibacterial action to a certain extent. It is well known that after initial reduction of the total bacterial load in periodontal pockets, the number of bacteria increases again in the weeks and months after treatment.[33, 34] In places, the counts were higher than baseline. The low baseline counts may be a result of more intensive attention paid to oral hygiene by the patients before entering the study.

The use of antibiotics is accompanied by increased risk of resistant strains developing and possible drug interactions, and therefore requires strict diagnosis criteria to be met.[35] HA was discussed as a possible alternative for the treatment of bacterial diseases by Pirnazar et al.[36] Their in vitro experiments showed bacteriostatic effects of HA against all six tested bacterial strains (including *A. actinomycetemcomitans* and *P. gingivalis*). Moreover, Carlson et al.[37] detected a growth inhibiting effect of up to 76.8% ± 3.7% of an organic matrix consisting of HA acting on pathogenic bacteria such as staphylococci, streptococci and *Pseudomonas aeruginos*a in orthopaedic infections.

When HA was applied once a week in vivo, no influence was seen on the counts of periodontopathogenic bacteria.[22] The aim of this study was to apply HA gels adjunctively to SRP and in daily supportive care over two weeks. More intensive application of HA may overcome some problems. For instance, the constant crevicular fluid flow rate of up to 40 µl per hour[38] is responsible for a rapid clearance of each subgingivally administered drug. In addition, it may be assumed that the amount of HA available is further reduced by bacterial hyaluronidases. Certain bacterial species *e.g. T. denticola*, have a hyaluronidase action.[39] Consequently, saturation of the bacterial hyaluronidases, which are needed to break through the physiological HA network, may have prevented bacterial spread.[37] Furthermore, effective pre-treatment of periodontitis patients prior to SRP may have some influence. The baseline examination in our study took place immediately before SRP. Here, beside low levels of clinical inflammatory markers (BOP) and laboratory variables (MPO, NE),

periodontopathogen counts were not high. HA seemed to be able to stabilize these low counts for a longer period and prevent the early regrowth of these bacterial species.

HA may have an immunomodulatory effect on polymorphonuclear leukocytes (PMNs). Together with fibronectin, HA stimulates PMN migration.[40] Further, HA improves the functions of PMNs in vitro and in vivo.[41] HA has been shown to suppress the release of superoxides from activated neutrophils.[42] In our study, a significant influence on neutrophil enzymes was not found. One reason may be patient treatment before SRP, resulting in low baseline values at the commencement of the study. On the other hand, another study analyzing the effect of a hyaluronate-carboxymethylcellulose membrane also did not detect an influence on PMN function.[43]

HA is a candidate for use in the restoration of periodontal integrity due to its complex interactions with the extracellular matrix and its components.[44, 45]. High molecular weight HA reduces proliferation of fibroblasts and lymphocytes in the epithelium in periodontal lesions.[46] In dogs, HA functions as a scaffold promoting adhesion and the proliferation of periodontal ligament cells; it is under discussion as a suitable scaffold incorporating selected molecules for clinical application in periodontal tissue regeneration.[47]

Notwithstanding its limitations (no placebo), our study indicates possible antibacterial effects of high molecular weight HA on periodontopathogenic bacteria as an adjunct to SRP, and also possibly increased probing depth reduction. Further studies are needed to verify the mode of action of HA in periodontitis patients.

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Figure 1.

Flowchart (adapted to Moher et al.)[28] of the study analysing the effect of hyaluronic acid (HA) as an adjunct after scaling and root planing

Figure 2.

Activities of myeloperoxidase (MPO) and neutrophil elastase (NE) at baseline as well as three and six months after SRP with (test) and without (control) the additional use of hyaluronan-containing gels (median, 10, 25, 50, 75, 90 percentiles and outliers)

Figure 3.

Counts of periodontopathogenic bacteria at baseline as well as three and six months after SRP with (test) and without (control) the additional use of hyaluronan-containing gels (median, 10, 25, 50, 75, 90 percentiles and outliers)

Table 1. Clinical results at baseline

Variable	Test group (n=17) mean±SD	Control group (n=17) mean±SD	U-test (p)
Mean age (years)	54.82±9.35	54.06±9.81	
Range (years)	42 - 70	41 - 72	
Male (n)	8	6	
Female (n)	9	11	
PD (mm)	4.2±0.4	4.1±0.4	0.235
Sites with PD ≥ 5 mm (n)	29 ±19	24±17	0.133
Sites with AL ≥ 5 mm (n)	83±18	88±14	0.218
AL (mm)	5.5±0.9	5.7±0.6	0.158
BOP (%)	16.3±8.7	18.8±11.1	0.642
API (%)	21±12	22±10	0.959

Table 2. Changes (Δ) of clinical data after three (t1) and six months (t2) in comparison with baseline (t0)

Variable	Test group (n=17)		Control group (n=17)		U-test (p)
	mean±SD	Wilcoxon test (p)	mean±SD	Wilcoxon test (p)	
ΔPD t1-t0 (mm)	-1.08±0.30	<0.001	-0.74±0.40	<0.001	0.014
ΔPD t2-t0 (mm)	-1.07±0.36	<0.001	-0.82±0.36	<0.001	0.046
ΔPD≥5mm t1-t0 (n)	-20±9	<0.001	-14±15	<0.001	0.021
ΔPD≥5mm t2-t0 (n)	-21±11	<0.001	-15±13	<0.001	0.045
ΔAL t1-t0 (mm)	-1.27± 0.63	<0.001	-1.00±0.62	<0.001	0.196
ΔAL t2-t0 (mm)	-1.24± 0.58	<0.001	-1.34±0.57	<0.001	0.547
Δ of sites with AL≥5mm t1-t0 (n)	-51±13	<0.001	-34.4±21	<0.001	0.013
Δ of sites with AL≥5mm t2-t0 (n)	-50±17	<0.001	-54±24	0.001	0.518
ΔBOP t1-t0 (%)	0.02±9.42	0.877	0.99±12.02	0.796	0.863
ΔBOP t2-t0 (%)	7.46±24.73	0.041	5.18±19.33	0.140	0.917
Δ API t1-t0 (%)	0±9	0.977	6±9	0.017	0.084
Δ API t2-t0 (%)	-3±8	0.103	0±11	0.814	0.395

Table 3. Prevalence of six periodontopathogens at baseline as well as three and six months after SRP in the test and control group

Species		Test group (n=17)	Control group (n=17)
A. actinomycetemcomitans			
	Baseline	6 (35%)	8 (47%)
	3 months	6 (35%)	4 (24%)
	6 months	4 (24%)*	13 (76%)*
P. gingivalis			
	Baseline	10 (59%)	8 (47%)
	3 months	10 (59%)	9 (53%)
	6 months	10 (59%)	13 (76%)
T. forsythia			
	Baseline	14 (82%)	11 (65)
	3 months	13 (76%)	11 (65%)
	6 months	17 (100%)	17 (100%)
T. denticola			
	Baseline	6 (35%)	6 (35%)
	3 months	5 (29%)	2 (12%) #
	6 months	3 (18%) #	3 (18%)
P. intermedia			
	Baseline	1 (6%)	1 (6%)
	3 months	5 (29%)	2 (12%)
	6 months	4 (24%)	6 (35%) #
C. rectus			
	Baseline	7 (33%)	7 (41%)
	3 months	8 (39%)	9 (53%)
	6 months	2 (11%)*#	12 (71%)*

* significant inter-group difference, $p < 0.05$

\# significant difference to baseline, $p < 0.05$

[**] PCP-UNC 15, Hu-Friedy Manufacturing Co., Chicago, IL, USA

[††] Periopaper; Oraflow Inc., Smithtown, New York, USA

[‡‡] ISO 60, Roeko GmbH, Langenau, Germany

[§§] Ultracain D-S, Sanofi-Aventis, Frankfurt/Main, Germany

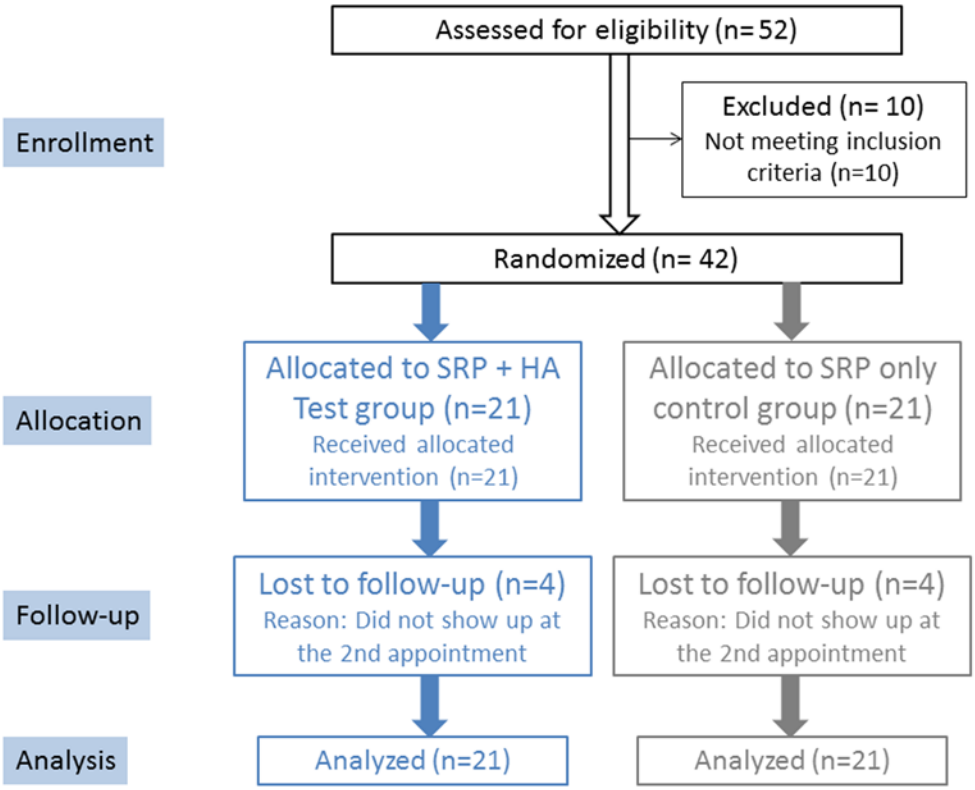
[***] 0.2% CHX, Curaden, Stutensee, Switzerland

[†††] Gengigel prof®, Merz Dental, Lütjenburg, Germany

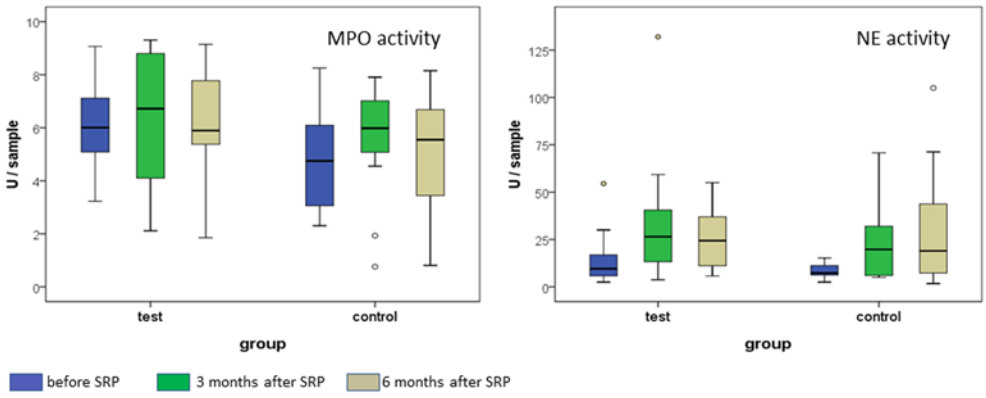
[‡‡‡] Gengigel®, Merz Dental, Lütjenburg, Germany

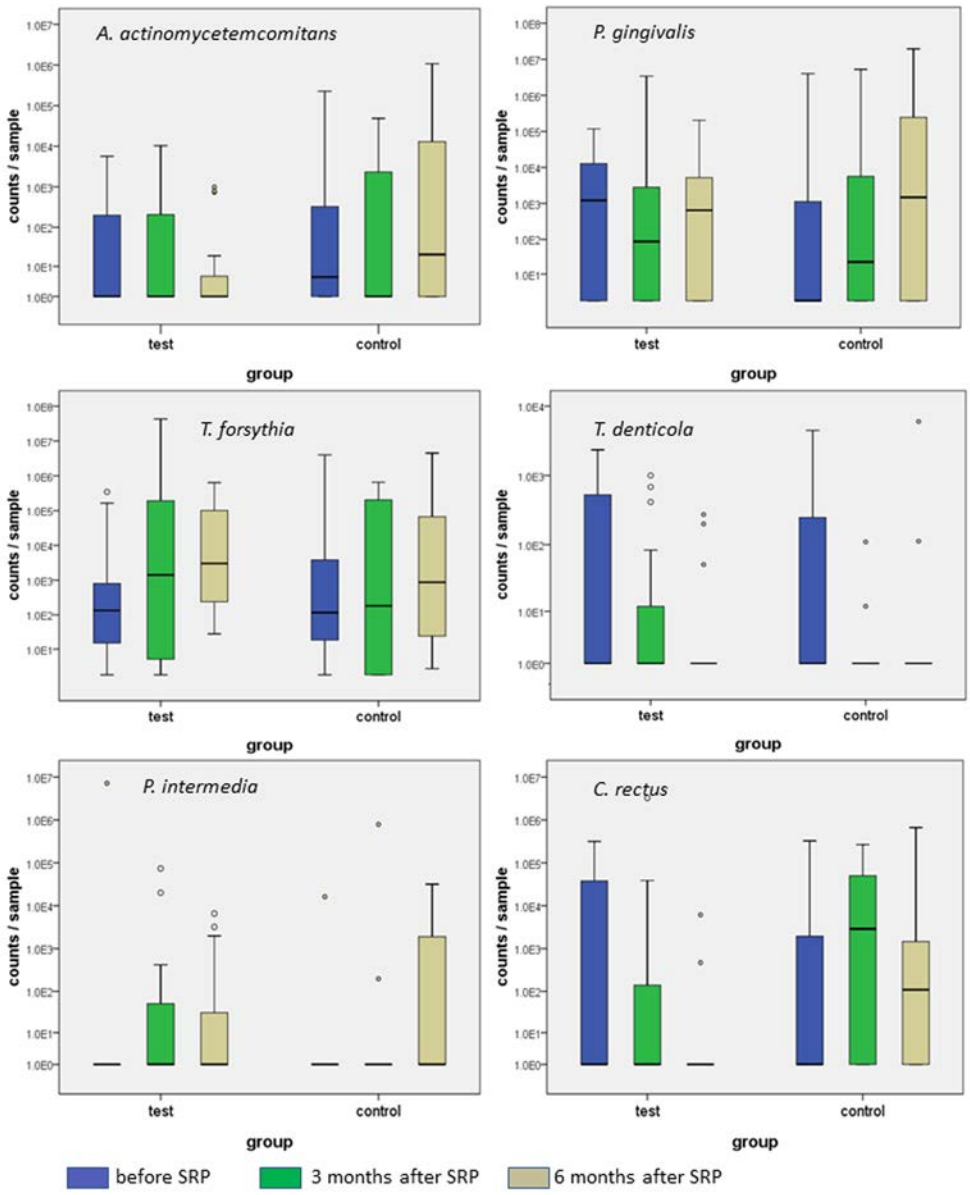
[§§§] Genomic Mini Kit®,A & A Biotechnology, Gdynia, Poland

[****] SPSS® Statistics 17.0, IBM Corporation, New York, NY, USA


Assessed for eligibility (n= 52)
Excluded (n= 10)
Not meeting inclusion criteria (n=10)
Randomized (n= 42)
Enrollment
Allocation
Follow-up
Analysis
Allocated to SRP + HA Test group (n=21)
Received allocated intervention (n=21)
Allocated to SRP only control group (n=21)
Received allocated intervention (n=21)
Lost to follow-up (n=4)
Reason: Did not show up at the 2nd appointment
Lost to follow-up (n=4)
Reason: Did not show up at the 2nd appointment
Analyzed (n=21)
Analyzed (n=21)

 DOI: 10.1902/jop.2012.120269




A. actinomycetemcomitans
P. gingivalis
T. forsythia
T. denticola
P. intermedia
C. rectus
counts / sample
test
control
group
before SRP
3 months after SRP
6 months after SRP