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Mechanical non-surgical treatment of peri-implantitis: a single-blinded randomized longitudinal clinical study. II. Microbiological results

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Abstract

Background: Peri-implantitis is common in patients with dental implants. We performed a single-blinded longitudinal randomized study to assess the effects of mechanical debridement on the peri-implant microbiota in peri-implantitis lesions.

Materials and Methods: An expanded checkerboard DNA–DNA hybridization assay encompassing 79 different microorganisms was used to study bacterial counts before and during 6 months following mechanical treatment of peri-implantitis in 17 cases treated with curettes and 14 cases treated with an ultrasonic device. Statistics included non-parametric tests and GLM multivariate analysis with $p<0001$ indicating significance and 80% power.

Results: At selected implant test sites, the most prevalent bacteria were: *Fusobacterium nucleatum* sp., *Staphylococci* sp., *Aggregatibacter actinomycetemcomitans*, *Helicobacter pylori*, and *Tannerella forsythia*. 30 min. after treatment with curettes, *A. actinomycetemcomitans* (serotype a), *Lactobacillus acidophilus*, *Streptococcus anginosus*, and *Veillonella parvula* were found at lower counts ($p<0.001$). No such differences were found for implants treated with the ultrasonic device. Inconsistent changes occurred following the first week. No microbiological differences between baseline and 6-month samples were found for any species or between treatment study methods in peri-implantitis.

Conclusions: Both methods failed to eliminate or reduce bacterial counts in peri-implantitis. No group differences were found in the ability to reduce the microbiota in peri-implantitis.

Key words: microbiota; peri-implantitis; *S. aureus*; treatment

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Conflict of interest and source of funding statement

Disclosure of conflict of interest: With regard to any process or product used to perform the present study, none of the authors have a conflict of interest.

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Biological complications in implant dentistry are referred to as peri-implant mucositis or peri-implantitis. Peri-implant mucositis has been defined as an inflammatory condition residing in the mucosa, and without clinical evidence of bone loss. Peri-implantitis also affects the surrounding bone and taking into consideration the alveolar bone remodelling occurring following implant placement (Lindhe et al. 2008).

Current data suggest that the prevalence of peri-implant mucositis is high. The prevalence of peri-implantitis may, at the subject level, be >30% (for a review, see Zitzmann & Berglundh 2008). There is a need to, comprehensively, assess the microbiota at titanium dental implants and to compare different treatment modalities.

A complex bacterial biofilm with a large variety of microorganisms develops

on all types of surfaces (Lamont & Jenkinson 2000). It is generally perceived that many different bacterial species are important in the aetiology of peri-implant mucositis and peri-implantitis (i.e. Pontoriero et al. 1994, Augthun & Conrads 1997, Salcetti et al. 1997, Leonardt et al. 1999, Quirynen et al. 2002, Renvert et al. 2007).

The oral and pharyngeal region is a significant source of microorganisms in humans.

Haemophilus influenzae, *Bacteroides*, and *Streptococci* species are commonly found in the oral–pharyngeal area (Chi et al. 2003, Swidsinski et al. 2007). *Staphylococcus aureus*, viridans *Streptococci*, and *Corynebacterium* species can also be found in the oral–pharyngeal area of healthy subjects (Konno et al. 2006). A link between the presence of bacteria in the oral and pharyngeal area and oral health status has recently been demonstrated for *Streptococci*, *Staphylococci*, *Candida*, *Pseudomonas*, and black-pigmented *Bacteroides* species (Ishikawa et al. 2008).

Therapies proposed for the management of peri-implant diseases are currently based on the evidence available from the treatment of periodontitis. In a systematic review, the authors concluded that mechanical non-surgical treatment of peri-implant mucositis lesions might be effective but not in cases of peri-implantitis. The data supporting this were, however, scarce (Renvert et al. 2008a). The treatment of infected dental implants is difficult and may require implant removal (Pye et al. 2009).

Because of technical difficulties in decontamination of infected dental implants without surgical intervention, adjunctive antibiotic therapy has been proposed (Mombelli et al. 2001). Results following non-surgical debridement combined with adjunct administration of local minocycline in the treatment of peri-implantitis have shown some effects on the clinical conditions (Persson et al. 2006, Renvert et al. 2006). Only marginal effects on the microbiota around implants treated with adjunct minocycline were demonstrated. Repeated applications of local antibiotics may be necessary to obtain both anti-bacterial and clinical effects (Renvert et al. 2008b).

The clinical effects of two mechanical therapeutic modalities in the management of cases with evidence of peri-implantitis have been evaluated in a 6-month clinical study demonstrating some reduction in bleeding on probing but with clinically non-relevant probing pocket depth (PPD) reduction (Renvert et al. 2009). We now report on the microbiological changes over time.

The aims of the present study were (1) to assess the microbiota at implants with a diagnosis of peri-implantitis and (2) to assess whether non-surgical mechanical treatment of peri-implantitis lesions, performed with titanium curettes, or by an ultrasound device specifically designed for the treatment of titanium implants, results in microbiological differences by these treatment modalities over time. Thus, the null hypothesis was that (1) therapy had no effects on the microbiota and (II) that there were no differences in the microbiological outcomes between the two treatment groups.

Materials and Methods

The Ethics Committee of Lund University, Sweden, approved the study. All subjects enrolled signed written informed consent. The CONSORT guidelines for clinical trials were followed. Subjects were enrolled if they presented with at least one dental implant with bone loss identified on intra-oral radiographs >2.5 mm and having a PPD $\geqslant 4$ mm with bleeding and/or pus on probing using a 0.2 N probing force. Subjects could have more than one implant but only one implant in each subject meeting the inclusion criteria was studied. In the case of more than one such implant, only the implant with the most severe condition was studied (all implants with peri-implantitis were treated).

The study was conducted between March 2007 and June 2008 at the University of Kristianstad, Sweden (clinical study component), and at the University of Bern, Switzerland (microbiological analysis). The following criteria were used to exclude subjects from entering the study: (I) poorly controlled diabetes mellitus (HbA1c $\geqslant 6.5$), (II) use of anti-inflammatory prescription medications, or antibiotics within the preceding 3 months, and (III) identified bone loss >2.5 mm in comparison with findings from radiographs taken immediately following placement of the implant supra-structure.

In the event that any subject had clinical evidence of periodontitis, such subjects were included if periodontal lesions at the remaining teeth had first been treated. Subjects without evidence of periodontitis were included without periodontal therapy. All included subjects must have at least one dental titanium implant meeting the diagnostic criteria for peri-implantitis (Lindhe et al. 2008). Subjects were randomly assigned to one of the two treatment regimens. The randomized allocation was carried out using a computer software program (SPSS Inc., Chicago, IL, USA). When performing their respective study tasks, the study examiner and the therapist were not jointly present with the study subject. Study subjects were instructed not to discuss the therapy with the study examiner. The study examiner was, therefore, unaware of study treatment allocation. The study examiner performed all clinical measurements and collected samples for microbiological analysis. The clinician performing treatment had >7 years of clinical experience in the mechanical treatment of implants with a diagnosis of peri-implantitis. The treatment code was not revealed until all microbiological assays had been completed and the electronic file (SPSS data file) had been established.

Adverse events (bleeding, pain, swelling) following therapy were evaluated using a questionnaire at the 1-month examination.

Checkerboard DNA–DNA hybridization, microbiological sampling, and analysis

Before any tissue manipulation, subgingival bacterial samples were taken immediately before debridement of the titanium implant at 30 min. after completion of the procedure, at 1 week, 1, 3 months, and at 6 months after the treatment. The area to be sampled was first isolated with cotton rolls to prevent contamination with saliva. Samples were taken with two sterile endodontic paper points size 55 (Absorbent Paper Points, Dentsply/Maillefer, Ballaigues, Switzerland) at the site of the treated titanium implant with the deepest probing depth. The paper points were simultaneously inserted and kept in situ for 20 s. Efforts were made to insert the paper points to the bottom of the space between the implant and the soft tissue lining. Size 55 endodontic paper points provide stability to insert the paper point into pockets around dental implants, and have been used previously in our studies on implant microbiology (Gerber et al.

2006). The collected paper points were placed in dry Eppendorf tubes (1.5 ml natural flat cap DNAse and RNAse-free microcentrifuge tubes, Starlab, Ahrensburg, Germany). All samples were stored in batches at -20°C and forwarded on a monthly basis on dry ice by overnight carrier to the oral microbiology laboratory at the University of Bern, Switzerland. At the laboratory, 0.15 ml Tris EDTA buffer (10 mM Tris-HCL, 1.0 mM EDTA, pH 7.6) and 0.10 ml 0.5 M NaOH were added to each eppendorf tube. The tubes were vortexed for 20 s. The paper points were then removed. The remaining content was pipetted onto slots and processed as described for the checkerboard DNA–DNA hybridization method (Socransky et al. 1994, 2004, Agerbaek et al. 2006, Persson et al. 2009). The species assessed by the checkerboard DNA–DNA hybridization method are presented (Table 1).

In order to obtain a fully detailed account of the identified bacteria, the digitized information was analysed using a software program (ImageQuant, Amersham Pharmacia, Piscataway, NJ, USA) allowing comparison of signals against standard lanes of known bacterial amounts (10^4 and 10^5 cells) in the appropriate checkerboard slot. Signals were converted to absolute counts by comparisons with these standards, and studied as the proportion of sites defined as having $\geqslant 1.0 \times 10^4$ and $\geqslant 1.0 \times 10^5$ bacterial cells. Cross-reactivity was routinely tested in the microbiology laboratory between known pure bacterial reference strains purchased or obtained from other laboratories (Table 1). Our quality control results were consistent with those reported elsewhere (Socransky et al. 2004). This also applied to the second panel of additional species studied. Thus, our analysis failed to identify measurable amounts of bacteria that could be explained as examples of cross-reactivity. The oral microbiology laboratory at the University of Bern, Switzerland, is certified by the Swiss Health Authorities (Bundesamt für Gesundheit, Bern, Switzerland) to conduct clinical and laboratory microbiological diagnostics.

Treatment procedures

Dental titanium implants in group 1 were treated with mechanical debridement using titanium curettes (Deppeler SA, Rolle, Switzerland). Implants in group 2 were treated with mechanical debridement using an ultrasonic device (LM Instruments Oy, Parainen, Finland) and with a specially designed tip for the treatment of infections around implants (LM Instruments Oy). All implants were polished with rubber cups and polishing paste. If needed, local anaesthesia was administered. At all study time points, subjects received oral hygiene instructions as necessary. Oral hygiene instructions were given specifically to manage plaque accumulation at implant sites using a soft toothbrush, inter-proximal brushes, toothpicks, or dental floss at each time point when subjects were examined. Likewise, and consistent with good clinical dental practice, oral hygiene instructions were given to enhance oral hygiene at natural teeth. This was also performed at each clinical visit.

Statistical methods

Power calculation

In the absence of reliable data on changes in the clinical conditions with regard to treatment, we assumed a mean difference of 0.6 mm in probing depth. This resulted in a sample size estimation of 18 subjects in each group. In the absence of microbiological data, we assumed a 40% difference between methods. This also resulted in a sample size estimation of 18 subjects in each group. Assuming that treatment would yield at least a 30% reduction in the bacterial counts (independent of the method), 36 subjects in the study would provide statistical power at the 80% level.

In order to assess microbiological changes within each treatment modality, the statistical analysis was performed using Kruskal–Wallis ANOVA and by repeat Mann–Whitney *U*-tests. General linear model multivariate analysis was used to assess the effects of treatment modality and time on the changes in the bacterial counts for the different species studied. The statistical software package SPSS 16.0 was used for the analysis (SPSS Inc.). Because of the number of variables included in the assays, significance was set at the $p<0.001$ level. Statistical differences at the $p<0.01$ were identified as trends of change.

Results

A total of 37 subjects were enrolled. Two subjects in group 1 and four subjects in group 2 discontinued the study. Three subjects discontinued after therapy and refused to return for recall visits. One subject discontinued after the 1-month follow-up due to the fact that he moved away from the area. Two subjects failed to return for the final 6-month examination (Fig. 1). Thus, complete data were available for 17 subjects in the group treated with hand instruments and 14 subjects in the group treated with the ultrasonic device. Seven subjects in each group were women and a total of five subjects (15.6%), three in group 1 and two in group 2, reported a smoking habit. None of the smokers changed their habit during the 6-month trial or had changed their smoking habits within the preceding 6 months.

Details of the clinical data and lack of statistically significant changes as effects of the mechanical therapies performed in the study are reported elsewhere (Renvert et al. 2009). Full-mouth bleeding on probing assessed at six sites per tooth remained at approximately 30% at all time points. Plaque scores decreased from 91% at dental titanium implants in group 1 to 37% at month 6 and from 82% to 38% in group 2. Similar changes in the plaque index scores were found at teeth. Comparing baseline with all other study time points, statistical analysis failed to demonstrate differences in PPD that were clinically relevant (Renvert et al. 2009). In this study, no adverse events occurred.

The mean PPD at the implant sites from which bacterial samples were collected was 5.1 mm (SD $\pm$ 0.6) in study group 1 and 5.2 mm (SD $\pm$ 0.7) in study group 2 (NS). At month 6, the corresponding values were 4.9 mm (SD $\pm$ 0.8) and 4.9 mm (SD $\pm$ 0.9), respectively (NS). Between baseline and month 6, statistical analysis failed to demonstrate differences in PPD change at the sites from which bacterial samples were collected. In addition, other dental titanium implants in subjects with implants who were diagnosed with peri-implantitis were treated using the same method as for the dental implant from which samples were taken. At baseline, all implants presented with bleeding on probing in group 1. At month 6, three of these implants presented with no evidence of bleeding on probing. All implants in group 2 also presented with bleeding on probing. At month 6, two of these implants presented with no evidence of bleeding.

Table 1. Bacteria included in the Checkerboard DNA–DNA hybridization assays

Bacteria	Collection	Bacteria	Collection
Aggregatibacter actinomycetemcomitans (a)	ATCC29523	*Alloscardovia omnicolens*	GUH071026
Aggregatibacter actinomycetemcomitans (Y4)	ATCC43718	*Actinomyces neuii*	GUH550898
Actinomyces israelii	ATCC 1201	*Aerococcus christensenii*	GUH070938
Actinomyces naeslundii	ATCC121045	*Anaerococcus vaginalis*	GUH290486
Actinomyces odontolyticus	ATCC17929	*Atopobium parvulum*	GUH160323
Capnocytophaga gingivalis	ATCC33612	*Atopobium vaginae*	GUH010535
Capnocytophaga ochracea	ATCC33596	*Bacteroides ureolyticus*	GUH080189
Capnocytophaga sputigena	ATCC33612	*Bifidobacterium bifidum*	GUH070962
Campylobacter gracilis	ATCC33236	*Bifidobacterium breve*	GUH080484
Campylobacter rectus	ATCC33238	*Bifidobacterium longum*	GUH180689
Campylobacter showae	ATCC451146	*Corynebacterium nigricans*	GUH450453
Eikenella corrodens	ATCC238345	*Corynebacterium aurimucosum*	GUH071035
Eubacterium saburreum	ASTCC33271	*Dialister* sp.	GUH071035
Fusobacterium nucl. naviforme	ASTCC49256	*Enterococcus faecalis*	GUH170812
Fusobacterium nucl. nucleatum	ATCC25586	*Enterococcus faecalis*	ATCC29212
Fusobacterium nucl. polymorphum	ATCC10953	*Echerichia coli*	GUH070903
Fusobacterium periodonticum	ATCC33993	*Gardnerella vaginalis*	GUH080585
Lactobacillus acidophilus	ATCC11975	*Haemophilus influenzae*	ATCC49247
Leptothrichia buccalis	ATCC14201	*Helicobacter pylori*	ATCC43504
Neisseria mucosa	ATCC33270	*Lactobacillus crispatus*	GUH160342
Parvimonas micra	ATCC19696	*Lactobacillus gasseri*	GUH170856
Prevotella intermedia	ATCC25611	*Lactobacillus iners*	GUH160334
Prevotella melaninogenica	ATCC25845	*Lactobacillus jensenii*	GUH160339
Prevotella nigrescens	ATCC33563	*Lactobacillusvaginalis*	GUH0780928
Porphyromonas gingivalis	ATCC33277	*Mobiluncus curtisii*	GUH070927
Propionybacterium acnes	ATCC11827/28	*Mobiluncus mulieris*	GUH070926
Selenomonas noxia	ATCC43541	*Peptoniphilus sp.*	GUH550970
Streptococcus anginosus	ATCC33397	*Peptostreptococcus anaerobius*	GUH160362
Streptococcus constellatus	ATCC27823	*Porphyromonas endodontalis*	ATCC35406
Streptococcus gordonii	ATCC10558	*Prevotella bivia*	GUH450429
Streptococcus intermedius	ATCC27335	*Prevotella disiens*	GUH190184
Streptococcus mitis	ATCC49456	*Proteus mirabilis*	GUH070918
Streptococcus mutans	ATCC25175	*Pseudomomas aeruginosa*	ATCC33467
Streptococcus oralis	ATCC35037	*Staphylococcus aureus*	ATCC25923
Streptococcus sanguinis	ATCC10556	*Staphylococcus aureus yellow strain*	GUH070921
Tannerella forsythia	ATCC43037	*Staphylococcus aureus white strain*	GUH070922
Treponema denticola	ATCC354405	*Staphylococcus epidermis*	DSMZ20044
Treponema socranskii	D40DR2	*Staphylococcus haemolyticus*	DSMZ20263
Veillonella parvula	ATCC10790	*Streptococcus agalactiae*	GUH230282
		Varibaculum cambriense	GUH070917

ATCC, American Type Culture Collection; D, sample from Forsyth Institute, Boston MA; GUH, Ghent University Hospital Collection, Ghent, Belgium; DSMZ, The German Resource Center for Biological Materials, Braunschweig Germany.

Baseline microbiological findings

The proportional distributions of bacteria identified as being positive based on bacterial detection levels of $> 1.0 \times 10^4$ and 1.0×10^5 bacterial cells in the two study groups are presented (Table 2). The most prevalent bacteria were *Aggregatibacter actinomycetemcomitans* Y4 (serotype b), *Fusobacterium nucleatum* sp., *Helicobacter pylori*, *Staphylococci* sp., and *Tannerella forsythia*. At baseline, statistically higher counts of bacterial species ($p < 0.001$) were found for 3/79 species [*A. actinomycetemcomitans* (serotype a), *Capnocytophaga sputigena*, and *Streptococcus gordonii*] in samples from implants to be treated in the hand instrument group in comparison with samples from implants to be treated in the ultrasonic treatment group.

Microbiological changes over time at implant sites treated with hand instruments

Statistical analysis demonstrated that between baseline samples and samples taken 30 min. after treatment, decreases in the counts of bacterial species were significant ($p < 0.001$) for *A. actinomycetemcomitans* (serotype a), *Lactobacillus acidophilus*, *Streptococcus anginosus*, and *Veillonella parvula*. Trends of a reduction in the bacterial counts ($p < 0.01$) were found for *C. sputigena*, *Lactobacillus buccalis*, *Streptococcus mutans*, *Streptococcus oralis*, and *Streptococcus sanguinis*. Statistical analysis failed to demonstrate other differences in the bacterial loads of the other bacterial species included in the microbiological analysis. At the $p < 0.001$ level, statistical analysis failed to demonstrate differences in the counts of bacterial species between baseline and 1-week samples. A trend of lower counts at week 1 was found for *Treponema denticola* ($p = 0.013$).

Statistical analysis demonstrated significantly lower counts of bacterial species at 1 month compared with the baseline values for *S. anginosus* ($p < 0.001$). Statistical analysis also found a trend of lower counts of bacterial species at 1 month for *L. acidophilus* ($p < 0.01$).

L. acidophilus and *S. anginosus* were found at significantly lower counts

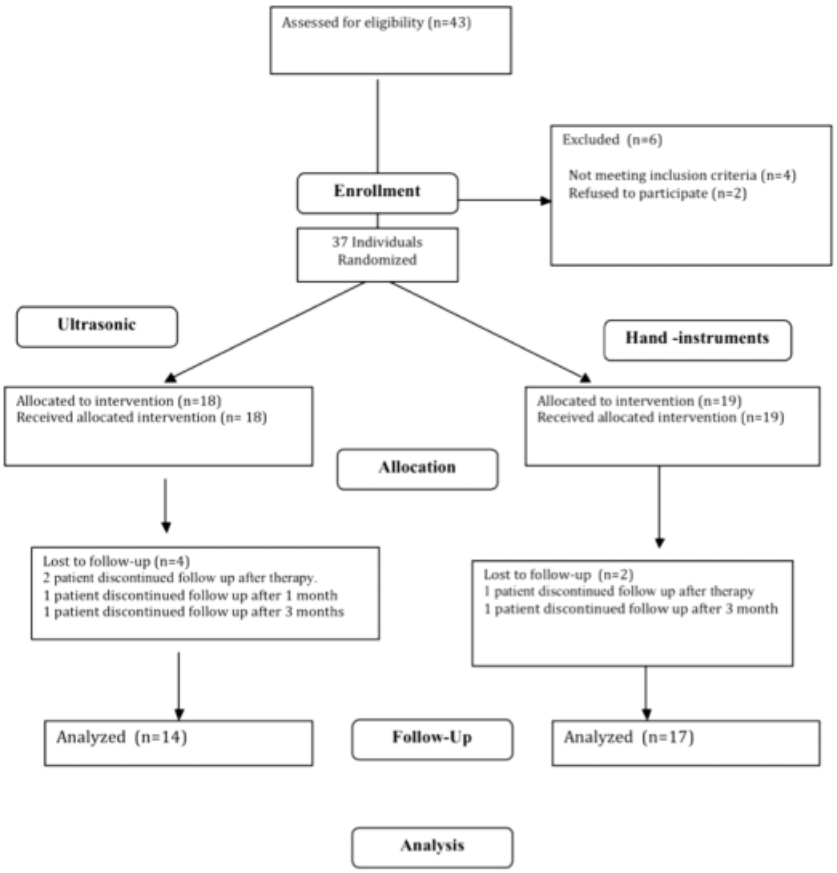

Fig. 1. A CONSORT E flowchart of the enrolment, allocation, follow-up, and analysis.

($p < 0.001$) at month 3 as compared with the baseline microbial values. Trends of lower bacterial counts at the $p < 0.01$ level at month 3 were also found for *A. actinomycetemcomitans* Y4 (serotype b). Statistical analysis by Kruskal–Wallis ANOVA failed to demonstrate differences in the bacterial counts (Tables 3 and 4).

Microbiological changes at implant sites treated with the ultrasonic device

Statistical analysis failed to demonstrate that the treatment resulted in changes between baseline and 30 min. post-treatment samples for any of the bacterial species studied.

Comparison between baseline and 1-week microbiological counts demonstrated a trend of lower counts for *Enterococcus faecalis* ($p = 0.022$). Between baseline and 1 month and baseline and 6 months, statistical analysis failed to demonstrate any differences between the samples at different time points. Analysis by Kruskal–Wallis ANOVA failed to demonstrate any differences, by time and treatment, for any of the bacterial species studied (Tables 3 and 4).

Differences in microbiological counts at implant sites treated with hand instruments or with the ultrasonic device

At none of the time points following interventions could statistically significant differences be identified for any of the species studied between implants treated with hand instruments or the ultrasonic device, and furthermore, with no trends of differences at $p- < 0.01$. The distributions of different *Staphylococci* species and *Pseudomonas aeruginosa* at the different study time points are presented in box-plot diagrams for subjects treated with hand instruments and with the ultrasonic device. With regard to bacterial counts, GL multivariate analysis failed to demonstrate a significant difference as an effect of time, subject, or treatment performed. The 25th, 50th, and 75th percentiles of select bacteria counts in the groups treated with hand instruments (group 1) and the group treated with the ultrasonic device (group 2) are presented for baseline and 6-month values (Table 5).

Intent-to-treat analysis

Among the 43 subjects assessed for eligibility, 19 subjects were allocated to be treated with hand instruments and 18 were allocated to be treated with an ultrasonic device (Fig. 1). During the study, two subjects in group 1 and four subjects in group 2 (the ultrasonic group) were lost to follow-up. In the intent-to-treat analysis, the latest available microbiological data were entered for each individual lost to follow-up assessments. Analysis by Kruskal–Wallis ANOVA failed to demonstrate differences over time for individual bacterial counts for all species included in the study within treatment arms. These findings were confirmed by repeat Mann–Whitney *U*-tests between baseline values and those obtained at the different time points. Furthermore, at each time point, no differences were found in the bacterial counts between treatment groups.

Discussion

The present study demonstrated that the clinical changes in both PPD and bleeding on probing between baseline and 6 months after treatment suggested limited clinical improvements and with no treatment group differences. The present study demonstrated that mechanical efforts with hand instruments designed for the treatment of dental titanium implants or with an ultrasonic device designed for the treatment of dental titanium implants, and oral hygiene instructions as deemed necessary did not result in important changes in the microbiota in pockets around implants with a diagnosis of peri-implantitis. Trends of bacterial reductions were soon lost, and 6 months after therapy, conditions were similar to those before treatment. These findings are consistent with the clinical data demonstrating limited clinical impact of therapy (Renvert et al. 2009).

Several factors may explain the limited clinical outcomes of therapy. It is likely that the instruments available for debridement around implants are not properly designed to reach the affected area. Because of implant design, location, and clinical conditions, it may be

Table 2. Counts of bacterial species at baseline defined as the proportion of sites positive for the microorganism studied at $\geqslant 1.0 \times 10^4$ and $\geqslant 1.0 \times 10^5$ bacterial cell detection levels

Bacteria	$\geqslant 1.0 \times 10^4$	$\geqslant 1.0 \times 10^5$		$\geqslant 1.0 \times 10^4$	$\geqslant 1.0 \times 10^5$
A. actinomycetemcomitans (a) 3	31.2	0.0	*A. neuii*	16.7	3.3
A. actinomycetemcomitans (Y)	50.0	6.2	*A, christensenii*	10.0	0.0
A. israelii	21.9	3.1	*A. vaginalis*	23.3	10.0
A. naeslundii	22.6	0.0	*A. parvulum*	20.0	0.0
A. odontolyticus	28.8	0.0	*A. vaginae*	26.7	10.0
C. gingivalis	15.6	3.1	*B. ureolyticus*	26.7	3.3
C. ochracea	34.6	3.1	*B. biavatii*	13.3	0.0
C. sputigena	21.9	3.1	*B. bifidum*	13.3	0.0
C. gracilis	12.5	3.1	*B. breve*	16.7	0.0
C. rectus	37.5	3.1	*B. longum*	13.3	6.7
C. showae	25.0	0.0	*C. nigricans*	10.0	0.0
E. corrodens	21.9	3.1	*C. aurimucosum*	20.0	0.0
E. saburreum	34.6	0.0	*Dialister* sp.	16.7	3.3
F. nucl. naviforme	31.2	6.2	*E. faecalis Ghent*	16.7	3.3
F. nucl. nucleatum	62.5	6.2	*E. faecalis ATCC*	6.7	0.0
F. nucl. polymorphum	40.6	3.1	*E. coli*	13.3	6.7
F. periodonticum	53.1	3.1	*G. vaginalis*	30.0	3.3
L. acidophilus	21.9	3.1	*H. influenzae*	33.7	3.3
L. buccalis	28.1	3.1	*H. pylori*	36.7	6.7
N. mucosa	31.2	3.1	*L. crispatus*	23.3	6.7
P. micra	25.0	3.1	*L. gasseri*	16.7	3.3
P. intermedia	31.2	3.1	*L. iners*	20.0	3.3
P. melaninogenica	31.2	6.2	*L. jensenii*	30.0	3.3
P. nigrescens	15.6	0.0	*L. vaginalis*	30.0	6.7
P. gingivalis	18.8	0.0	*M. curtisii*	16.7	0.0
P. acnes	15.6	0.0	*M. mulieris*	13.3	3.3
S. noxia	15.6	0.0	*Peptoniphilus*	26.7	0.0
S. anginosus	28.1	3.1	*P. anaerobius*	20.0	0.0
S. constellatus	19.4	0.0	*P. endodontalis*	13.3	6.7
S. gordonii	31.2	3.1	*P. bivia*	20.0	0.0
S. intermedius	18.8	3.1	*P. disiens*	16.7	0.0
S. mitis	15.6	3.1	*P. mirabilis*	13.3	3.3
S. mutans	28.1	3.1	*P. aeruginosa*	10.0	3.3
S. oralis	18.2	3.1	*S. aureus*	31.2	9.4
S. sanguinis	15.6	3.1	*S. aureus yellow strain*	26.7	6.7
T. forsythia	21.9	12.5	*S. aureus white strain*	23.3	3.3
T. denticola	18.6	3.1	*S. epidermis*	20.0	3.3
T. socranskii	34.4	3.1	*S. haemolyticus*	26.7	3.3
V. parvula	40.6	3.1	*S. agalactiae*	20.0	3.3
			V. cambriense	23.3	3.3

very difficult to provide adequate debridement of dental implants.

Furthermore, the clinical data demonstrated that although oral hygiene improved, the efficacy of oral hygiene efforts did not meet the expected standards. Thus, the design of implants and placement of the dental titanium implants and their superstructures might have made it difficult to maintain a plaque- and inflammation-free environment around the dental titanium implants studied. The development of a biofilm at dental titanium implants with peri-implantitis may specifically include pathogens that are therapy resistant.

Few studies have assessed a broader selection of bacterial species in periodontal conditions than a selection of 40 individual species suggested by several authors (Socransky et al. 1994, 2004, Li et al. 2004, Paju et al. 2009, Torrungruang et al. 2009). There are, however, a large variety of bacteria to be identified in the oral cavity (Paster et al. 2006, Konno et al. 2006). Although the microbiota in gingivitis and periodontitis has been investigated extensively, the information on bacteria around implants with a diagnosis of peri-implantitis is limited. The present study demonstrated that different members of the genus *Staphylococcus* can be found at dental titanium implants with a diagnosis of peri-implantitis and with a prevalence that is higher than the prevalence of bacteria such as *T. forsythia* and *Porphyromonas gingivalis*. The natural habitat for *Staphylococci* includes skin, mucous membranes, and also the oral cavity and the upper respiratory tract (Lederer et al. 2007). Although most *Staphylococci* are part of the commensal bacteria, they may become severe pathogens in combination with trauma or implantation of medical devices (Cook et al. 2009, Fujimura et al. 2009). It is well established that *S. aureus* has a high affinity to titanium surfaces (Gristina 1994, Harris et al. 2006, 2007, Gaudreau et al. 2009, Khoo et al. 2009). In the medical field, orthopaedic implant-related bacterial infections have been associated with high morbidity that may lead to limb amputation, with *S. aureus* as the dominant cause of such infections (Lauderdale et al. 2010). In vitro studies have shown that scraping an implant surface fails to remove or collect *S. aureus* from a biofilm development and that standard culture methods therefore are negative for *S. aureus* (Bjerkan et al. 2009).

Table 3. Counts of bacterial species at month 6 (panel 1) defined as the proportion of sites positive for the microorganism at $\geqslant 1.0 \times 10^4$ and $\geqslant 1.0 \times 10^5$ bacterial cell detection levels

Bacteria	Hand instrument $\geqslant 1.0 \times 10^4$	Hand instrument $\geqslant 1.0 \times 10^5$	Ultrasonic $\geqslant 1.0 \times 10^4$	Ultrasonic $\geqslant 1.0 \times 10^5$
A. actinomycetemcomitans (a)	38.7	9.7	26.9	3.8
A. actinomycetemcomitans (Y)	48.4	3.2	38.5	7.7
A. israelii	25.8	3.2	26.9	3.8
A. naeslundii	32.3	3.2	23.1	7.7
A. odontolyticus	25.8	3.2	19.2	0.0
C. gingivalis	32.3	3.2	15.4	0.0
C. ochracea	48.4	16.1	30.8	7.7
C. sputigena	32.3	9.7	30.8	3.8
C. gracilis	35.5	9.7	30.8	0.0
C. rectus	54.8	6.5	26.9	0.0
C. showae	35.5	3.2	19.2	0.0
E. corrodens	32.3	3.2	26.9	7.7
E. saburreum	38.7	3.2	23.1	3.8
F. nucl. naviforme	58.1	12.9	46.2	7.7
F. nucl. nucleatum	81.0	12.9	53.8	7.7
F. nucl. polymorphum	48.4	6.5	34.6	3.8
F. periodonticum	79.0	9.7	34.6	3.8
L. acidophilus	29.0	3.2	23.1	3.8
L. buccalis	55.2	6.5	30.8	7.7
N. mucosa	35.5	12.9	30.8	11.5
P. micra	35.5	9.7	23.1	0.0
P. intermedia	58.1	3.2	34.6	0.0
P. melaninogenica	54.8	6.5	19.2	0.0
P. nigrescens	25.8	3.2	15.4	0.0
P. gingivalis	29.0	3.2	19.2	0.0
P. acnes	29.0	3.2	19.1	0.0
S. noxia	35.5	3.2	19.8	0.0
S. anginosus	35.5	3.2	15.4	0.0
S. constellatus	16.1	3.2	19.2	0.0
S. gordonii	45.2	6.5	19.2	0.0
S. intermedius	32.3	3.2	15.4	0.0
S. mitis	41.9	3.2	23.1	0.0
S. mutans	45.2	12.9	19.2	0.0
S. oralis	29.0	6.5	15.6	0.0
S. sanguinis	22.6	3.2	11.5	0.0
T. forsythia	38.7	3.2	54.4	23.1
T. denticola	29.0	3.2	30.8	0.0
T. socranskii	51.6	6.5	30.8	0.0
V. parvula	55.8	16.1	34.6	3.8

S. aureus is a coagulase-positive opportunistic pathogen commonly found in post-operative wounds. Nasal carriage of the methicillin-resistant *S. aureus* (MRSA) is common in adults, and the nasal prevalence (Choi et al. 2006) is similar to what has been found at implant sites with peri-implantitis in the present study. In a recent study, *S. aureus* was identified in aggressive periodontitis (Fritschi et al. 2008). Other studies have shown that *S. aureus* is prevalent at titanium implants with varying degrees of inflammation (Renvert et al. 2008c). If *S. aureus* has been identified at implant sites within 3 months after the implants have been inserted, the likelihood that *S. aureus* will remain at 12 months is considerable (Salvi et al. 2008). Between baseline and month 6 in the present study, both the mechanical therapy using hand instruments and ultrasound treatment failed to change the proportion of *S. aureus* (three different subspecies), *S. epidermis*, and *S. haemolyticus*. Data suggest that the absence of protective antibody titres to *S. aureus* may be explanatory in dental implant failures (Kronström et al. 2000, 2001). Further studies are therefore needed to assess the role of *S. aureus* as well as other pathogens currently not routinely assessed at infected dental titanium implants.

One of the problems with the antibacterial therapy of *S. aureus* is also an increased risk for colonization of *P. aeruginosa* (Ratjen et al. 2001). *S. aureus* and *P .aeruginosa* are commonly found on orthopaedic implants (Arcioloa et al. 2005). In the present study, *P. aeruginosa* was found at some of the dental titanium implants both at baseline and at month 6. *P. aeruginosa* has been found in oral samples but the information on *P. aeruginosa* in the oral cavity is limited (Fritschi et al. 2008). Data suggest that *P. aeruginosa* can also be retrieved from dental impressions (Egusa et al. 2008). It appears that bacteria associated with periodontitis can enhance the ability of *P. aeruginosa* to invade respiratory epithelial cells (Pan et al. 2009). In other studies of dental implants diagnosed with peri-implantitis, high rates of *A. actinomycetemcomitans*, *Fusobacterium* spp., *P. gingivalis*, *P. aeruginosa*, and *T. forsythia* have been found (Van de Velde et al. 2009). Thus, the symbiosis between *Bacteroides* species and *P. aeruginosa* may enhance the persistence of *P. aeruginosa* in subgingival

Table 4. Bacterial counts at month 6 (panel 2) defined as the proportion of sites positive for the microorganism at $\geqslant 1.0 \times 10^4$ and $\geqslant 1.0 \times 10^5$ bacterial cell levels

Bacteria	Hand instrument $\geqslant 1.0 \times 10^4$	Hand instrument $\geqslant 1.0 \times 10^5$	Ultrasonic $\geqslant 1.0 \times 10^4$	Ultrasonic $\geqslant 1.0 \times 10^5$
A. neuii	12.9	0.0	8.0	0.0
A, christensenii	19.4	0.0	16.0	0.0
A. vaginalis	25.8	3.2	16.0	0.0
A. parvulum	16.1	3.2	0.0	0.0
A. vaginae	35.5	19.4	35.0	24.0
B. ureolyticus	25.8	3.2	40.0	0.0
B. biavatii	16.1	0.0	12.0	0.0
B. bifidum	16.1	0.0	16.0	0.0
B. breve	19.3	0.0	12.0	0.0
B. longum	22.6	0.0	16.0	0.0
C. nigricans	25.8	0.0	16.0	4.0
C. aurimucosum	12.9	0.0	12.0	0.0
Dialister sp.	16.1	0.0	20.0	0.0
E. faecalis (GUH)	16.1	0.0	8.0	0.0
E. faecalis (ATCC)	9.7	0.0	4.0	0.0
E. coli	16.1	3.2	28.0	16.0
G. vaginalis	35.5	6.5	36.0	4.0
H. influenzae	29.7	9.7	53.0	4.0
H. pylori	58.9	9.7	56.0	12.0
L. crispatus	38.7	3.2	36.0	0.0
L. gasseri	29.0	3.2	16.0	0.0
L. iners	32.3	3.2	23.0	0.0
L. jensenii	28.7	3.2	32.0	4.0
L. vaginalis	35.5	3.2	20.0	4.0
M. curtisii	16.1	0.0	16.0	0.0
M. mulieris	9.7	0.0	16.0	0.0
Peptoniphilus	25.8	0.0	4.0	0.0
P. anaerobius	22.6	0.0	24.0	0.0
P. endodontalis	32.3	0.0	24.0	0.0
P. bivia	22.6	0.0	16.0	0.0
P. disiens	22.6	0.0	20.0	0.0
P. mirabilis	29.0	3.2	20.0	8.0
P. aeruginosa	19.4	0.0	20.0	4.0
S. aureus	29.0	6.5	26.9	3.8
S. aureus yellow strain	16.1	0.0	20.0	0.0
S. aureus white strain	35.5	0.0	16.0	0.0
S. epidermis	29.0	3.2	24.0	0.0
S. haemolyticus	32.3	3.2	36.0	0.0
S. agalactiae	16.1	3.2	16.0	0.0
V. cambriense	22.6	0.0	16.0	0.0

lesions when inflamed and infected tissues are not surgically removed.

The intent-to-treat analysis carrying forward the latest known values for subjects who never completed the study further confirmed that neither treatment modality had an impact on the bacterial counts for any of the species studied. This strengthens our perception that not even shortly after treatment could a relevant reduction in the counts of bacterial species be obtained by either of these two treatment modalities.

Although both treatment arms seemed to reduce the counts of bacterial species commonly associated with periodontitis (i.e. *A. actinomycetemcomitans* Y4 (serotype b), *P. gingivalis*, *T. forsythia*, and *T. denticola*) during the first weeks after the treatments, these effects disappeared within a few weeks. This may point to the difficulties in the debridement of the implant surfaces, and that these pathogens remain at the sites of infection. It is of interest that the mechanical therapy with the hand instrument appeared to be more efficient in reducing the counts of bacterial species at the 30 min. post-treatment time point, whereas the counts of bacterial species remained similar immediately after treatment in the group treated with the ultrasonic device. At 1 week, however, the ultrasonic treatment seemed to have reduced the counts of some of the bacterial species.

The proportion of sites with positive counts of bacterial species at the 10^5 cut of level of bacterial counts was low, whereas at the 10^4 cut-off level, higher counts of bacterial species were found. This may be reflected by the fact that the paper points had been sterilized three times to increase the stiffness of the paper points. This may have reduced the fluid absorption capacity of the endodontic paper points such that bacteria would not aggregate on these. In previous studies, it has been shown that originally sterilized endodontic paper points yield higher bacterial counts than sampling with curettes at dental implant sites (Gerber et al. 2006).

The study may be considered as underpowered. However, the statistical power analysis of the previous study was based on anticipated changes for

Table 5. Results by hand instrument- and ultrasonic-treated groups presented as 25th, 50th, and 75th percentile values (all at values $\times 10^5$ bacterial cells) for selected bacteria at baseline and at month 6

Time	Percentile	*A. actinomycetemcomitans*		*P. gingivalis*		*T. forsythia*		*H. pylori*		*P. aeruginosa*		*S. aureus*	
		hand instr.	ultrasonic	hand instr.	ultrasonic	hand instr.	ultrasonic	hand instr.	ultrasonic	hand instr.	ultrasonic	hand instr.	ultrasonic
Baseline	25th	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50th	0.05	0.00	0.00	0.00	0.24	0.00	0.00	0.17	0.00	0.00	0.00	0.00
	75th	0.18	0.37	0.09	0.00	0.93	0.00	0.44	0.44	0.24	0.06	0.11	0.02
Month 6	25th	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50th	0.00	0.00	0.00	0.00	0.00	0.24	0.20	0.00	0.00	0.00	0.06	0.00
	75th	0.12	0.24	0.13	0.16	0.10	0.93	0.69	0.44	0.12	0.24	0.20	0.11

Instr., instrument.

the primary clinical study outcome parameters (Renvert et al. 2009). In the absence of microbiological data for bacteria specifically associated with peri-implantitis, we were unable to perform a similar power analysis for the microbiological data.

Conclusions

We failed to demonstrate differences in the counts of bacterial species studied within each study group over a follow-up period of 6 months.

We failed to demonstrate that the two treatment methods studied resulted in differences in the counts of the bacterial species studied.

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References

Agerbaek, M. R., Lang, N. P. & Persson, G. R. (2006) Comparisons of bacterial patterns present at implant and tooth sites in subjects on supportive periodontal therapy. I. Impact of clinical variables, gender and smoking. *Clinical Oral Implants Research* **17**, 18–24.

Arciola, C. R., An, Y. H., Campoccia, D., Donati, M. E. & Montanaro, L. (2005) Etiology of implant orthopedic infections: a survey on 1027 clinical isolates. *International Journal of Artificial Organs* **28**, 1091–1100.

Augthun, M. & Conrads, G. (1997) Microbial findings of deep peri-implant bone defects. *International Journal of Oral and Maxillofacial Implants* **12**, 106–112.

Bjerkan, G., Witsø, E. & Bergh., K. (2009) Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro. *Acta Orthopedica* **80**, 245–250.

Chi, D. H., Hendley, J. O., French, P., Arango, P., Hayden, F. G. & Winther, B. (2003) Nasopharyngeal reservoir of bacterial otitis media and sinusitis pathogens in adults during wellness and viral respiratory illness. *American Journal of Rhinology* **17**, 209–214.

Choi, C. S., Yin, C. S., Bakar, A. A., Sakewi, Z., Naing, N. N., Jamal, F. & Othman, N. (2006) Nasal carriage of *Staphylococcus aureus* among healthy adults. *Journal of Microbiology, Immunology and Infection* **39**, 458–464.

Cook, A., Berne, J. & Norwood, S. (2009) The incidence and complications of methicillin-resistant *Staphylococcus aureus* in a community Level I trauma center. *Journal of Trauma* **67**, 102–107.

Egusa, M. H., Watamoto, T., Matsumoto, T., Abe, K., Kobayashi, M., Akashi, Y. & Yatani, H. (2008) Clinical evaluation of the efficacy of removing microorganisms to disinfect patient-derived dental impressions. *International Journal of Prosthodontics* **21**, 531–538.

Fritschi, B. Z., Albert-Kiszely, A. & Persson, G. R. (2008) *Staphylococcus aureus* and other bacteria in untreated periodontitis. *Journal of Dental Research* **87**, 589–593.

Fujimura, S., Sato, T., Kikuchi, T., Zaini, J., Gomi, K. & Watanabe, A. (2009) Efficacy of clarithromycin plus vancomycin in mice with implant-related infection caused by biofilm-forming *Staphylococcus aureus*. *Journal of Orthopedic Science* **14**, 658–661.

Gaudreau, G., Costache, V., Houde, C., Cloutier, D., Montalin, L., Voisine, P. & Baillot, R. (2009) Recurrent sternal infection following treatment with negative pressure wound therapy and titanium transverse plate fixation. *European Journal of Cardiothoracic Surgery* (epub ahead of print).

Gerber, J., Wenaweser, D., Heitz-Mayfield, L., Lang, N. P. & Persson, G. R. (2006) Comparison of bacterial plaque samples from titanium implant and tooth surfaces by different methods. *Clinical Oral Implants Research* **17**, 1–7.

Gristina, A. G. (1994) Implant failure and the immuno-incompetent fibro-inflammatory zone. *Clinical Orthopaedic Related Research* **298**, 106–118.

Harris, L. G., Mead, L., Müller-Oberländer, E. & Richards, R. G. (2006) Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces. *Journal of Materials Science: Materials in Medicine A* **78**, 50–58.

Harris, L. G., Meredith, D. O., Eschbach, L. & Richards, R. G. (2007) *Staphylococcus aureus* adhesion to standard micro-rough and electropolished implant materials. *Journal of Materials Science: Materials in Medicine* **18**, 1151–1156.

Ishikawa, A., Yoneyama, T., Hirota, K., Miyake, Y. & Miyatake, K. (2008) Professional oral health care reduces the number of oropharyngeal bacteria. *Journal of Dental Research* **87**, 594–598.

Khoo, X., Hamilton, P., O'Toole, G. A., Snyder, B. D., Kenan, D. J. & Grinstaff, M. W. (2009) Directed assembly of PEGylated-peptide coatings for infection-resistant titanium metal. *Journal American Chemical Society* **131**, 10992–10997.

Konno, M., Baba, S., Mikawa, H., Hara, K., Matsumoto, F., Kaga, K., Nishimura, T., Kobayashi, T., Furuya, N., Moriyama, H., Okamoto, Y., Furukawa, M., Yamanaka, N., Matsushima, T., Yoshizawa, Y., Kohno, S., Kobayashi, K., Morikawa, A., Koizumi, S., Sunakawa, K., Inoue, M. & Ubukata, K. (2006) Study of upper respiratory tract bacterial flora: first report. Variations in upper

respiratory tract bacterial flora in patients with acute upper respiratory tract infection and healthy subjects and variations by subject age. *Journal of Infection and Chemotherapy* **12**, 83–96.

Kronström, M., Svensson, B., Erickson, E., Houston, L., Braham, P. & Persson, G. R. (2000) Humoral immunity host factors in subjects with failing or successful titanium dental implants. *Journal of Clinical Periodontology* **27**, 875–882.

Kronström, M., Svenson, B., Hellman, M. & Persson, G. R. (2001) Early implant failures in patients treated with Brånemark System titanium dental implants: a retrospective study. *International Journal of Oral Maxillofacial Implants* **16**, 201–207.

Lamont, R. J. & Jenkinson, H. F. (2000) Subgingival colonization by Porphyromonas gingivalis. *Oral Microbiology & Immunology* **15**, 341–349.

Lauderdale, K. J., Malone., C. L., Boles, B. R., Morcuende, J. & Horswill, A. R. (2010) Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. *Journal of Orthopedic Research.* **28**, 55–61.

Lederer, S. R., Riedelsdorf, G. & Schiffl, H. (2007) Nasal carriage of meticillin resistant *Staphylococcus aureus*: the prevalence, patients at risk and the effect of elimination on outcomes among outclinic haemodialysis patients. *European Journal of Medical Research* **26**, 284–288.

Leonardt, Å., Renvert, S. & Dahlén, G. (1999) Microbial findings at failing implants. *Clinical Oral Implants Research* **10**, 339–345.

Li, J., Helmerhorst, E. J., Leone, C. W., Troxler, R. F., Yaskell, T., Haffajee, A. D., Socransky, S. S. & Oppenheim, F. G. (2004) Identification of early microbial colonizers in human dental biofilm. *Journal of Applied Microbiology* **97**, 1311–1318.

Lindhe, J. & Meyle, J.Group D of European Workshop on Periodontology. (2008) Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *Journal of Clinical Periodontology* **35** (Suppl.), 282–285.

Mombelli, A., Feloutzis, A., Brägger, U. & Lang, N. P. (2001) Treatment of peri-implantitis by local delivery of tetracycline. Clinical, microbiological and radiological results. *Clinical Oral Implants Research* **12**, 287–294.

Paju, S., Pussinen, P. J., Suominen-Taipale, L., Hyvönen, M., Knuuttila, M. & Könönen, E. (2009) Detection of multiple pathogenic species in saliva is associated with periodontal infection in adults. *Journal of Clinical Microbiology* **47**, 235–238.

Pan, Y., Teng, D., Burke, A. C., Haase, E. M. & Scannapieco, F. A. (2009) Oral bacteria modulate invasion and induction of apoptosis in HEp-2 cells by *Pseudomonas aeruginosa*. *Microbial Pathogenesis* **46**, 73–79.

Paster, B. J., Olsen, I., Aas, J. A. & Dewhirst, F. E. (2006) The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontology 2000* **42**, 80–87.

Persson, G. R., Hitti, J., Verhelst, R., Vaneechoutte, M., Persson, R., Hirschi, R., Weibel, M., Rothen, M., Temmerman, M., Paul, K. & Eschenbach, D. (2009) The vaginal microflora in relation to gingivitis. *BioMed Central, Infectious Diseases* **22**, 6.

Persson, G. R., Salvi, G. E., Heitz-Mayfield, L. J. & Lang, N. P. (2006) Antimicrobial therapy using a local drug delivery system in the treatment of peri-implantitis. I: microbiological outcomes. *Clinical Oral Implants Research* **17**, 386–393.

Pontoriero, R., Tonetti, M. P., Carnevale, G., Mombelli, A., Nyman, S. R. & Lang, N. P. (1994) Experimentally induced peri-implant mucositis. A clinical study in humans. *Clinical Oral Implants Research* **5**, 254–259.

Pye, A. D., Lockhart, D. E., Dawson, M. P., Murray, C. A. & Smith, A. J. (2009) A review of dental implants and infection. *Journal of Hospital Infection* **72**, 104–110.

Quirynen, M., De Soete, M. & van Steenberghe, D. (2002) Infectious risks for oral implants: a review of the literature. *Clinical Oral Implants Research* **13**, 1–19.

Ratjen, F., Comes, G., Paul, K., Posselt, H. G., Wagner, T. O. & Harms, KGerman Board of the European Registry for Cystic Fibrosis (ERCF). (2001) Effect of continuous antistaphylococcal therapy on the rate of *P. aeruginosa* acquisition in patients with cystic fibrosis. *Pediatric Pulmonology* **31**, 13–16.

Renvert, S., Lessem, J., Dahlén, G., Lindahl, C. & Svensson, M. (2006) Topical minocycline microspheres versus topical chlorhexidine gel as an adjunct to mechanical debridement of incipient peri-implant infections: a randomized clinical trial. *Journal of Clinical Periodontology* **33**, 362–369.

Renvert, S., Lessem, J., Dahlén, G., Renvert, H. & Lindahl, C. (2008b) Mechanical and repeated antimicrobial therapy using a local drug delivery system in the treatment of peri-implantitis: a randomized clinical trial. *Journal of Periodontology* **79**, 836–844.

Renvert, S., Lindahl, C., Renvert, H. & Persson, G. R. (2008c) Clinical and microbiological analysis of subjects treated with Brånemark or AstraTech implants: a 7-year follow-up study. *Clinical Oral Implants Research* **19**, 342–347.

Renvert, S., Roos-Jansåker, A.-M. & Claffey, N. (2008a) Non-surgical treatment of peri-implant mucositis and peri-implantitis: a literature review. *Journal of Clinical Periodontology* **35**, 305–315.

Renvert, S., Roos-Jansåker, A. M., Lindahl, C., Renvert, H. & Persson, G. R. (2007) Infection at titanium implants with or without a clinical diagnosis of inflammation. *Clinical Oral Implants Research* **18**, 509–516.

Renvert, S., Samuelsson, E., Lindahl, C. & Persson, R. E. (2009) Mechanical non surgical treatment of peri-implantitis. A double blind randomized longitudinal clinical study – I Clinical results. *Journal of Clinical Periodontology* **36**, 604–609.

Salcetti, J. M., Moriarty, J. D., Cooper, L. F., Smith, F. W., Collins, J. G., Socransky, S. S. & Offenbacher, S. (1997) The clinical, microbial, and host response characteristics of the failing implant. *International Journal of Oral and Maxillofacial Implants* **12**, 32–42.

Salvi, G. E., Fürst, M. M., Lang, N. P. & Persson, G. R. (2008) One-year bacterial colonization patterns of *Staphylococcus aureus* and other bacteria at implants and adjacent teeth. *Clinical Oral Implants Research* **19**, 242–248.

Socransky, S. S., Haffajee, A. D., Smith, C., Martin, L., Haffajee, J. A., Uzel, N. G. & Goodson, J. M. (2004) Use of checkerboard DNA–DNA hybridization to study complex microbial ecosystems. *Oral Microbiology and Immunology* **29**, 352–362.

Socransky, S. S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) Checkerboard DNA–DNA hybridization. *Biotechniques* **17**, 788–792.

Swidsinski, A., Göktas, O., Bessler, C., Loening-Baucke, V., Hale, L. P., Andree, H., Weizenegger, M., Hölzl, M., Scherer, H. & Lochs, H. (2007) Spatial organisation of microbiota in quiescent adenoiditis and tonsillitis. *Journal of Clinical Patholology* **60**, 253–260.

Torrungruang, K., Bandhaya, P., Likittanasombat, K. & Grittayaphong, C. (2009) Relationship between the presence of certain bacterial pathogens and periodontal status of urban Thai adults. *Journal of Periodontology* **80**, 122–129.

Van de Velde, T., Thevissen, E., Persson, G. R., Johansson, C. & De Bruyn, H. (2009) Two-year outcome with nobel direct(R) implants: a retrospective radiographic and microbiologic study in 10 patients. *Clinical Implant and Dental Related Research* **11**, 183–193.

Zitzmann, N. U. & Berglundh, T. (2008) Definition and prevalence of periimplant diseases. *Journal of Clinical Periodontology* **35** (Suppl. 8), 286–291.

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Clinical Relevance

Scientific rationale for the study: Peri-implantitis is a complication in implant dentistry. Data on treatment efficacy are scarce.
Principal findings: The study showed that mechanical debridement alone with hand instruments equipped with titanium tips or with an ultrasonic device equipped with specific tips to be used at implants can neither improve clinical conditions nor effectively reduce bacterial counts at implants with peri-implantitis. Peri-implantitis infection includes several virulent microorganisms.
Clinical implications: In order to care for subjects with peri-implantitis lesions, other treatment modalities should be developed and tested.