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# Topical minocycline microspheres *versus* topical chlorhexidine gel as an adjunct to mechanical debridement of incipient peri-implant infections: a randomized clinical trial

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*Abstract*

**Aim:** This randomized clinical trial presents a 12-month follow-up of the clinical and microbiological results after application of minocycline microspheres as an adjunct to mechanical treatment of incipient peri-implant infections compared with an adjunctive treatment using 1% chlorhexidine gel application.

**Material and Methods:** Thirty-two subjects with probing depth ⩾4 mm, combined with bleeding and/or exudate on probing and presence of putative pathogenic bacteria were given oral hygiene instructions and mechanical treatment of infected areas adjacent to implants. The subjects were then randomly assigned adjunctive subgingival antimicrobial treatment using either chlorhexidine gel or minocycline microspheres. Sixteen patients in the minocycline group and 14 in the chlorhexidine group completed the study. Follow-up examinations were carried out after 10 days, 1, 2, 3, 6, 9 and 12 months.

**Results:** The adjunctive use of minocycline microspheres resulted in improvements of probing depths and bleeding scores, whereas the adjunctive use of chlorhexidine only resulted in limited reduction of bleeding scores. For the deepest sites of the treated implants in the minocycline group, the mean probing depth was reduced from 5.0 to 4.4 mm at 12 months. This study could not show any significant difference in the levels of bacterial species or groups at any time point between the two antimicrobial agents tested. The present findings encourage further studies on adjunctive use of minocycline microspheres in the treatment of peri-implant lesions.

**Conclusions:** The use of a local antibiotic as an adjunct to mechanical treatment of incipient peri-implantitis lesions demonstrated improvements in probing depths that were sustained over 12 months.

Key words: antiseptics; arestin; chlorhexidine; local antibiotics; minocycline; peri-implantitis; peri-implant mucositis; treatment

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The concept that bacteria play a major role in the aetiology of peri-implant mucositis and peri-implantitis is well documented (Berglundh et al. 1992, Pontoriero et al. 1994, Augthun & Conrads 1997, Salcetti et al. 1997, Mombelli & Lang 1998, Quirynen et al. 2002). Mombelli (2002) reviewed the role of bacteria in causation of peri-implantitis and found support for the concept that the microflora present in the oral cavity before implant placement influence the

microflora developing on implants. Several reports have indicated a healing potential of peri-implant tissues following suppression of the peri-implant microbiota by mechanical and chemical means (see reviews by Klinge et al. 2002; Mombelli 2002).

Owing to technical difficulties in decontaminating implants by mechanical means alone, the use of adjunctive antimicrobial components has been proposed for the treatment of peri-implant infection (Flemmig 1994, Kao et al. 1997, Lang et al. 1997). Topical application of chlorhexidine is one treatment modality that has been proposed. However, Porras et al. (2002) did not find additional improvements following use of topical chlorhexidine to supplement mechanical debridement as compared with mechanical debridement alone. Application of tetracycline fibres has also been recommended (Lang et al. 1997). This adjunctive treatment seems to have some support from the findings of Schenk et al. (1997) and Mombelli et al. (2001). In a recent publication by Büchter et al. (2004), a significantly greater gain in the mean probing attachment levels in peri-implantitis lesions treated with local application of a slow release doxycycline (Atridox®) was reported. Subjects treated with Atridox® also demonstrated a significant reduction in bleeding on probing ($0.27 \pm 0.06$, $p = 0.001$). Accordingly, the use of slow-release antibiotics as an adjunct to mechanical treatment of incipient peri-implantitis lesions seems to improve the healing results. Recently, an agent using microspheres containing minocycline hydrochloride (1 mg) was developed (Arestin®). Studies on this agent indicate clinically beneficial effects when used as an adjunct to supra- and subgingival mechanical debridement (Williams et al. 2001, Henderson et al. 2002, Oringer et al. 2002, Van Dyke et al. 2002, Paquette et al. 2003). In a previous publication evaluating the short-term (3 months) effect of Arestin® as an adjunct to mechanical treatment, Renvert et al. (2004) reported clinical improvements of incipient peri-implantitis lesions.

A

The aim of the present trial was to study the clinical and microbiological results during a period of 12 months after application of Arestin® as an adjunct to mechanical treatment of peri-implant infections compared with an adjunctive treatment using 1% chlorhexidine gel application.

B

## Materials and Methods

This is a single-centre, single-blind, randomized, two-arm clinical trial. The randomization was performed before initiation of the study. Sealed and numbered envelopes with the treatment code were securely kept in a safe deposit box. As the individuals were enrolled for treatment, the treating clinician opened the envelope with the same serial number as the patient, and the assigned treatment was given. The clinician performing the measurements was unaware of the treatment given.

C

### Subjects

Patients for this study were recruited from individuals examined in a survey evaluating the prevalence of mucositis/peri-implantitis 10–12 years following placement of Brånemark implants. Thirty-two patients, 41–75 years of age, meeting the following criteria were included:

- a minimum of one osseointegrated implant with loss of bone limited to $\leqslant 3$ threads comparing radiographs at the time of screening for this study with those taken following placement of the implant suprastructure 10–12 years previously;
- one or more peri-implant sites with probing depth $\geqslant 4$ mm, combined with bleeding and/or pus on probing using 0.2 N probing force; and
- results of microbial sample from the deepest site around the implant demonstrating: (1) occurrence of anaerobic bacteria present as detected by anaerobic culturing and (2) the presence of one ore more of the following species: *Porphyromonas gingivalis* , *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythensis*, *Actinobacillus actinomycetemcomitans* or *Treponema denticola*, detected by DNA probe analysis at a minimum of level 2 on a five-grade scale according to Papapanou et al. (1997).

Patients with any of the following conditions were excluded from the study:

- pregnant or lactating females, or females of child-bearing potential not using acceptable methods of birth control;
- medication within 1 month of the screening visit with agents known to affect periodontal status;
- requirement of prophylactic antibiotics for treatment; and
- use of systemic antibiotics within 3 months before the study, and allergy to tetracyclines.

C (cont.)

### Measurements

One and the same examiner, unaware of the treatment group for the patient, performed all the measurements.

D

*Full-mouth plaque score*

The presence of dental plaque along the gingival/mucosal margin recorded after use of disclosing dye and expressed as a percentage of examined sites within each patient (six sites per tooth and implant).

*Full-mouth bleeding score*

Bleeding appearing after measurement of probing depth and expressed as a percentage of examined sites (six sites per tooth and implant).

*Local plaque score*

The presence of dental plaque along the mucosal margin at four sites of each treated implant, recorded after use of disclosing dye and expressed as a percentage of implant sites within each patient.

*Probing depth*

Recorded at four sites of each treated implant to the nearest millimetre using a plastic probe with a standardized force of 0.2 N (Hawe Click-Probe, Hawe Neos Dental, Switzerland).

*Bleeding on microbial sampling*

Bleeding appearing at the mucosal margin after paper point sampling of the deepest site around each treated implant and expressed as a percentage of sampled implant sites within each patient.

*Bleeding on probing/microbial sampling*

A bleeding score for treated implants based upon bleeding appearing at the mucosal margin after paper-point sam-

A pling of the deepest site, and bleeding after recordings of probing depth at the other three sites, expressed as a percentage of implant sites within each patient. This combined probing/microbial sampling bleeding score was used, as microbial sampling of the deepest site was made before probing depth recordings and most likely would have affected the bleeding tendency at a subsequent probing of these sites.

*Microbial sampling*

The deepest site of each qualifying implant was isolated with cotton rolls. Supragingival plaque was removed with sterile cotton pellets. Four paper points were inserted submucosally until resistance was met and left for 20 s. The tips of two paper points were cut off with sterile scissors and dropped into a vial containing 3.3 ml reduced transport fluid VMGA III and used for microbiological culturing. The other two paper points were placed in a sterile dry Eppendorf tube to be used for DNA technique.

## Experimental Procedures

### Screening

B The ethics committee at Lund University approved the study. At the screening examination (performed 11–21 days before baseline/treatment), a signed written consent and a medical history including current medications were collected. Full-mouth plaque scores, full-mouth bleeding scores, full-mouth probing depths, local plaque scores, microbial samples and bleeding on probing/microbial sampling scores were obtained.

### Baseline

At baseline, assigned treatments were provided.

### Follow-up visits

C At days 10, 30, 60, 90,180, 270 and 360, local plaque scores, probing depths and bleeding on probing were recorded at each treated implant. Microbial samples were obtained at screening and at days 10, 30, 90, 180 and 360.

### Treatment

Oral hygiene instruction was provided, together with supra- and subgingival

C (cont.)

calculus and plaque removal from implant surfaces using scalers specially designed for implants (Hawe Neos deplaquer, Hawe Neos dental, Switzerland) and a rubber cup with polishing paste.

D Patients were randomly assigned to minocycline or chlorhexidine treatment, with 16 individuals in each group. Cards assigning adjunctive use of minocycline microspheres or chlorhexidine gel were randomly placed in sealed numbered envelopes. The envelopes were opened by the treating clinician and the assigned treatment was given to the patient. The examining clinician, responsible for recording clinical parameters and obtaining microbiological samples, were unaware of the treatment performed.

E

#### Minocycline treatment

Following cessation of bleeding and isolating/drying the implant to be treated, a single unit dose of Arestin® containing 1 mg minocycline (OraPharma Inc., Warminster, PA, USA) was inserted submucosally at each of the four sites around the implant with a special dispenser. Microspheres appearing at the mucosal margin were condensed submucosally using an appropriate instrument.

#### Chlorhexidine treatment

Following cessation of bleeding and isolating/drying the implant to be treated, approximately 1 ml of 1% chlorhexidine gel (Corsodyl, Glaxo SmithKline, Mölndal, Sweden) was inserted submucosally into each of the four sites around the implant using a disposable 2 ml syringe.

Patients were instructed not to brush their teeth/implants for 12 h and to avoid the use of inter-proximal cleaning devices for 10 days in the treated area. At the follow-up visits, additional oral hygiene advice was given to patients requesting such information. Apart from this, no supportive treatment was provided.

All treatments were carried out by one and the same clinician.

### Processing of the Bacterial Plaque Samples

A volume 100 μl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6) was added to the Eppendorf tubes and mixed, and subsequently another 100 μl 10.5 M NaOH was added and the suspension was boiled for 5 min. After boiling, 800 μl 5 M ammonium acetate was added to each tube and the samples were processed with the checkerboard methodology according to standardized procedures (Socransky et al. 1994, Papapanou et al. 1997). Immobilization of bacterial samples onto nylon membranes was performed by ultraviolet (UV) light and incubated at 120°C, and was completed within 4 weeks from sample collection.

Digoxigenin-labelled, whole genomic probes were prepared by random priming using the High-Prime labelling kit (Roch Diagnostic, Indianapolis, IN, USA) from the following 12 bacterial strains: *P. gingivalis* (FDC381), *P. intermedia* (ATCC 25611), *P. nigrescens* (ATCC 33563), *T. forsythensis* (ATCC43037), *A. actinomycetemcomitans* (FDC Y4), *Fusobacterium nucleatum* (ATCC 10953), *T. denticola* (OMGS 3271), *Micromonas* (formerly *Peptostreptococcus) micros* (OMGS 2852), *Campylobacter rectus* (ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Selenomonas noxia* (OMGS 3119) and *Streptococcus intermedius* (ATCC 27335).

The hybrids formed between the bacterial DNA and the probes were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubation with a chemiluminiscent substitute (CSPD, Roch Diagnostic). Evaluation of the signal was performed at a LumiImager™ workstation (Roch Diagnostic) by comparing the obtained signals with those of pooled standard samples containing $10^6$ (high-standard) or $10^5$ (low-standard) of each of the 12 bacterial species. The sensitivity and specificity of whole genomic probes constructed as above have been described previously (Socransky et al. 1994, 2004), and a comparison between checkerboard hybridization and culture in the identification of subgingival microbiota has also been published (Papapanou et al. 1997). In addition, the probes were cross-tested against the 12 species of the panel in order to distinguish cross-hybridizations. The chemiluminiscents signals obtained were transformed into a scale of scores from 0 to 5 according to Papapanou et al. (1997). Thus, 0 indicated no signal; 1, signal density weaker than the one of low standard ($< 10^5$); 2, signal density equal to the

one of the low standard (= $10^5$); 3, signal density higher than the one of low standard but lower than that of the high standard (< $10^6$); 4, signal density equal to the one of high standard (= $10^6$); and 5, signal density higher than the one of high standard (>$10^6$). The occurrence of individuals positive for each of the investigated bacterial species was described at the cut-off level of Score 2 corresponding to a level of $10^5$ cells.

### Culture analysis

The other two paper points transferred to the VMGA III vial were processed in the laboratory within 24 h. After suspension, the samples were diluted in VMG I and plated on selective and non-selective media. A volume of 0.1 ml of each dilution was inoculated on Brucella agar plates enriched with 5% defibrinated horse blood plus 0.5% haemolysed horse blood and 5 mg/l of menadione. The plates were thereafter incubated anaerobically for 7 days at 37°C in jars, using hydrogen combustion in 95% $H_2$ and 5% $CO_2$. In addition, one blood agar plate and one selective Enterococcosel agar (BBL, Microbiolgical Systems, Cockeysville, MD, USA) plate were aerobically incubated overnight.

The total viable count (TVC) was determined as the total number of colony-forming units (CFU) obtained on the anaerobically incubated Brucella agar plates. *P. gingivalis* and *P. intermedia/P. nigrescens* were identified on the Brucella agar plates by their capacity to form black-pigmented colonies. They were differentiated by testing their auto-fluorescence in UV light. Special attention was paid to the aerobically incubated blood agar plate for growth of enteric rods. They were enumerated, and further identified using the API 20E system. Enteroccoci were identified and enumerated on the selective medium by the ability to form black colonies on Enterococcosel agar.

#### Data analysis and statistics

The two treatment groups were compared with respect to various characteristics at screening using Fisher's exact tests for categorical variables and independent sample *t*-tests for continuous variables.

A The effects of treatment were compared with respect to plaque scores, probing depths and bleeding on probing/microbial sampling. The outcome measures were averaged within each subject, followed by comparisons between the two groups using independent sample *t*-tests.

For the microbiological data obtained by checkerboard analysis, a mean value for the different microorganisms of each patient was calculated. TVC were estimated by the number of CFU obtained from anaerobic non-selective Brucella-blood agar plate. Black-pigmented anaerobic species (*P. gingivalis* and *P. intermedia/P. nigrescens*), enteric rods and enterococci were calculated as % of TVC.

### Results

Two patients in the chlorhexidine group were excluded because of use of systemic antibiotics for treatment of other diseases, leaving 14 individuals in the chlorhexidine group and 16 in the minocycline group. Soreness in the gums as a result of treatment was reported at day 10 by one subject in the chlorhexidine group and by five subjects in the minocycline group. Patient characteristics at screening were not significantly different between the two groups for any of the tested variables (Table 1).

B The mean plaque scores at screening for all four sites of treated implants amounted to 45% and 50% for chlorhexidine and minocycline groups, respectively. The mean plaque scores were reduced and at 12 months the corresponding values were reduced to 21% and 27%. Compared with these C numbers, plaque scores for the deepest sites of each treated implant were higher throughout the study and although a reduction as compared with baseline was noted at 12 months, plaque was detected in 27% for implants treated with chlorhexidine and 48% for the implants treated with minocycline. However, no significant differences were observed for any of these two plaque scores between the chlorhexidine and minocycline groups at any of the time points during the study (Table 2).

D The mean probing depths at screening for all four sites of treated implants amounted to 3.9 mm for both the chlorhexidine and minocycline groups. At 12 months, the probing depth remained the same for the chlorhexidine group, while the depth was reduced to 3.6 mm for the minocycline group. Probing depth for the deepest sites at screening averaged 5.1 mm for the chlorhexidine group and 5.0 mm for the minocycline group. At 12 months, the corresponding depths were 4.9 and 4.4 mm, respectively. Reductions

*Table 1.* Patient characteristics at screening for chlorhexidine and minocycline groups (means ± SD; proportions; ranges within brackets)

| Characteristics | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) |
|---|---|---|
| Age | 61.1 ± 8.6 | 65.6 ± 8.6 |
| Gender (female/male) | 11/3 | 7/9 |
| Smoker (never/former/present) | 4/7/3 | 5/6/5 |
| Full-mouth plaque score (%) | 52 ± 25 | 52 ± 28 |
| Full-mouth bleeding score (%) | 55 ± 16 | 64 ± 19 |
| Number of treated implants | 2.6 (1–6) | 3.1 (1–5) |

E

*Table 2.* Mean plaque scores ± SD (%) throughout the 12-month observation interval for treated implants at all four sites per implant and at the deepest site of each implant for chlorhexidine and minocycline groups

| Observation interval | All four sites/implant | | Deepest site/implant | |
|---|---|---|---|---|
| | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) |
| Screening | 45 ± 27 | 50 ± 25 | 67 ± 39 | 76 ± 33 |
| 10 days | 46 ± 25 | 49 ± 19 | 67 ± 43 | 77 ± 34 |
| 1 month | 22 ± 18 | 17 ± 19 | 35 ± 35 | 32 ± 41 |
| 2 months | 22 ± 20 | 26 ± 23 | 35 ± 37 | 44 ± 42 |
| 3 months | 22 ± 20 | 26 ± 20 | 36 ± 41 | 42 ± 35 |
| 6 months | 31 ± 27 | 31 ± 24 | 49 ± 45 | 52 ± 41 |
| 9 months | 24 ± 20 | 28 ± 23 | 42 ± 44 | 60 ± 41 |
| 12 months | 21 ± 18 | 27 ± 24 | 27 ± 30 | 48 ± 44 |

A of probing depths from screening were significantly greater for the minocycline group than for the chlorhexidine group after 1, 2, 3, 6, 9 and 12 months both for scores at all four sites and for scores at deepest sites only (Table 3).

C Mean bleeding on probing/microbial sampling scores at screening for all four sites of treated implants amounted to 86% and 88% for the chlorhexidine and minocycline groups, respectively. At 12 months, the corresponding values were 78% and 71%. In the chlorhexidine group, 100% bleeding on microbial sampling was recorded after 12 months as compared with 86% for the minocycline group. Reductions of bleeding on probing/microbial sampling scores from screening were significantly greater for the minocycline group than for the chlorhexidine group after 1, 2, 3 and 6 months for all four sites of the treated implants. A comparison of bleeding on microbial sampling for the deepest sites between the two groups, in spite of numerical mean differences, revealed significant differences only after 1, 6 and 12 months (Table 4).

The microbiological analysis, as determined by the checkerboard technique, revealed similar longitudinal effects of the two antimicrobial agents. In Fig. 1, six species are shown (*A. actinomycetemcomitans, P. gingivalis, T. forsythensis, T. denticola, F. nucleatum* and *P. intermedia*). A similar pattern was also found for *Micromonas micros, E. corrodens, S. noxia, C. rectus, P. nigrescens* and *S. intermedius* (data not shown). For all the bacterial species investigated the mean values were the highest at visit one and gradually decreased during the experimental period. No statistical significance was obtained between the two antimicrobials for any bacteria and at any time point.

Using culture, the TVC showed a significant increase after 6–12 months and reached a maximal mean value of $10^6$ cells/ml of transport medium (Fig. 2). This increase was similar in the two groups. Regarding *P. gingivalis*, a significant drop of the viable count was found after treatment in both groups and remained at a very low level in the minocycline group (<0.2% of TVC) during the 1-year experimental period.

Both enteric rods and enterococci were recovered on several occasions, however, in low percentages of TVC. However, at visit three (after the antimicrobial treatment), enteric rods showed a slightly and temporarily higher level (not statistically significant) in both treatments groups.

B *Table 3.* Mean probing depths ± SD (mm) throughout the 12-month observation interval for treated implants at all four sites per implant and at the deepest site of each implant for chlorhexidine and minocycline groups

| Observation interval | All four sites/implant | | Deepest site/implant | |
|---|---|---|---|---|
| | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) |
| Screening | 3.9 ± 0.3 | 3.9 ± 0.7 | 5.1 ± 0.5 | 5.0 ± 0.9 |
| 10 days | 3.7 ± 0.3 | 3.8 ± 0.7 | 5.0 ± 0.6 | 4.8 ± 1.0 |
| 1 month | 3.7 ± 0.3 | 3.6 ± 0.7** | 4.8 ± 0.7 | 4.4 ± 0.9* |
| 2 months | 3.8 ± 0.3 | 3.5 ± 0.7** | 4.9 ± 0.7 | 4.2 ± 0.8** |
| 3 months | 3.9 ± 0.3 | 3.5 ± 0.6*** | 5.0 ± 0.7 | 4.1 ± 0.8*** |
| 6 months | 3.9 ± 0.4 | 3.6 ± 0.6** | 5.0 ± 0.7 | 4.3 ± 0.7** |
| 9 months | 3.9 ± 0.4 | 3.6 ± 0.7** | 4.9 ± 0.6 | 4.4 ± 0.8* |
| 12 months | 3.9 ± 0.4 | 3.6 ± 0.6*** | 4.9 ± 0.6 | 4.4 ± 0.7** |

Reductions from screening significantly greater for the minocycline group than for the chlorhexidine group (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).

D *Table 4.* Mean bleeding on probing/microbial sampling scores ± SD (%) throughout the 12-month observation interval for treated implants at all four sites per implant and at the deepest site of each implant for chlorhexidine and minocycline groups

| Observation interval | All four sites/implant | | Deepest site/implant | |
|---|---|---|---|---|
| | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) | Chlorhexidine ($N = 14$) | Minocycline ($N = 16$) |
| Screening | 86 ± 14 | 88 ± 12 | 100 ± 0 | 100 ± 0 |
| 10 days | 69 ± 24 | 56 ± 24 | 74 ± 34 | 58 ± 43 |
| 1 month | 63 ± 24 | 40 ± 16** | 71 ± 42 | 42 ± 34* |
| 2 months | 62 ± 17 | 38 ± 21*** | 71 ± 37 | 60 ± 37 |
| 3 months | 71 ± 14 | 45 ± 26** | 73 ± 35 | 57 ± 35 |
| 6 months | 79 ± 14 | 55 ± 25** | 87 ± 29 | 58 ± 33* |
| 9 months | 72 ± 18 | 62 ± 24 | 85 ± 30 | 73 ± 36 |
| 12 months | 78 ± 13 | 71 ± 22 | 100 ± 0 | 86 ± 23* |

Reductions from screening significantly greater for the minocycline group than for the chlorhexidine group (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$).
At the deepest site, bleeding was assessed after microbial sampling, while bleeding at the other three implant sites was assessed after probing.

## Discussion

The patients of this comparative trial showed peri-implant lesions not exceeding three threads of bone loss at the implants selected for study. Surgical therapy and open debridement was not considered a first choice for these lesions. It was anticipated that closed mechanical debridement combined with topical antimicrobial treatment should lead to some or adequate improvement. An agent using microspheres containing minocycline was compared with the use of chlorhexidine gel as an adjunct to the mechanical treatment. Chlorhexidine was selected based on previous recommendations (Flemmig 1994, Kao et al. 1997, Lang et al. 1997).

In a pharmacokinetic study of locally delivered minocycline microspheres (Paquette et al. 2000), it was concluded that subgingival administration of minocycline microspheres (1 mg minocycline) resulted in detectable levels of minocycline in the serum but at levels lower than would be expected from a similar oral dose by a factor of four to five. Saliva levels were far higher (by factor $10^3$) and were still present in some subjects after 14 days, indicating a sustained release of minocycline from the minocycline microspheres.

Improvements of the levels of mucosal inflammation and probing depth in incipient peri-implant lesions following mechanical or combined mechanical/antimicrobial treatment have previously been accomplished as evidenced from some case reports (Braß & Anil 1991, Ciancio et al. 1995, Mombelli et al. 2001) and a few comparative studies

(Schenk et al. 1997, Porras et al. 2002) (see a review by Roos-Jansåker et al. 2003, and recently Büchter et al. 2004). A comparison between the results of the above studies and the results of the present trial, however, is difficult to make because of the uncertainties about the nature of the lesions before treatment in the various studies.

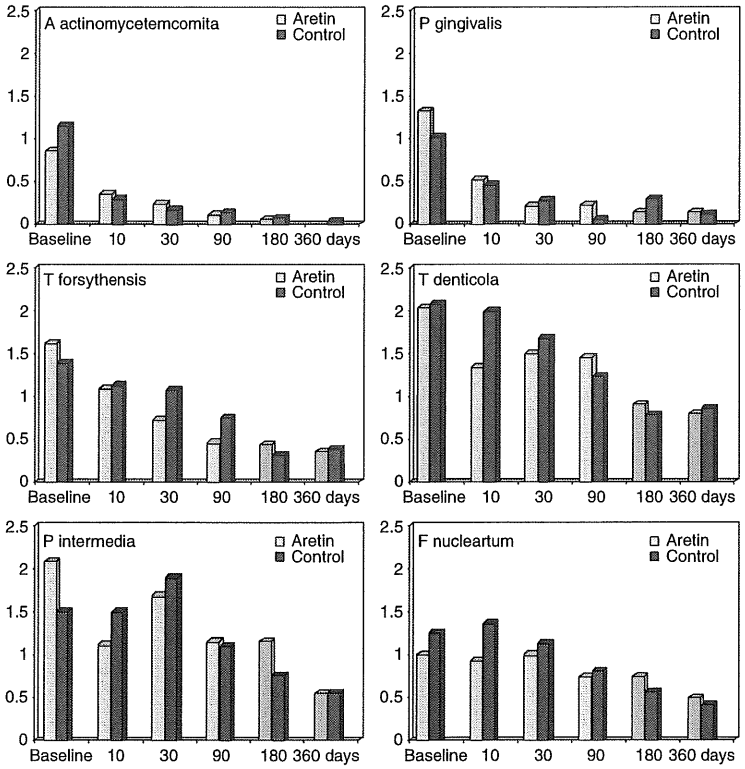


*Fig 1.* Mean checkerboard score of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythensis, Treponema denticola, Prevotella intermedia* and *Fusobacterium nucleatum* in topical minocycline microspheres (Arestin®)-treated peri-implantitis sites *versus* topical chlorhexidine for 12 months.

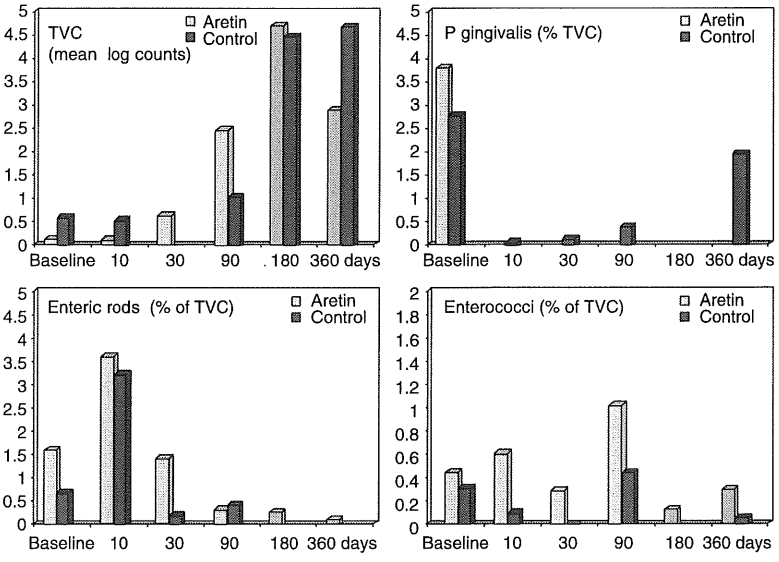


*Fig 2.* Mean log counts of total viable counts (TVC) and % of TVC for *Porphyromonas gingivalis*, enteric rods and enterococci after culture in topical minocycline microspheres (Arestin®)-treated peri-implantitis sites *versus* topical chlorhexidine for 12 months.

The results over 12 months of the present study demonstrated similar, but moderate, improvements of the plaque scores at the treated implants for both study groups, especially for the scores at the deepest sites of the implants. This may be related to the fact that plaque control was not systematically reinforced during the study.

The combined mechanical/antimicrobial treatment for the chlorhexidine group did not result in any reduction in probing depth and only limited reduction of bleeding scores. In part, these results corroborate the findings by Porras et al. (2002), who did not find any adjunctive effects of local 0.12% chlorhexidine irrigation+submucosal 0.12% chlorhexidine gel application+10 days of 0.12% chlorhexidine mouthrinse. These results suggest that chlorhexidine applied as rinses and gels may have limited antimicrobial effects in peri-implant lesions. Inter-dental cleaning was avoided for the first 10 days in both groups. This may have been more negative for the chlorhexidine group because of the low substantivity of the chlorhexidine gel.

The results of the microbiological analysis show an expected pattern generally; however, there are some interesting notes to be commented on. The patients showed an overall low prevalence and level of the target bacteria as compared with advanced periodontitis cases (Renvert et al. 1996). The selected patients were required to have the presence of one or more of the following species: *P. gingivalis* , *P. intermedia* , *P. nigrescens, T. forsythensis, A. actinomycetemcomitans* or *T. denticola*, detected by DNA probe analysis at a minimum of level 2 on a five-grade scale according to Papapanou et al. (1997). It is possible that we would have found more pronounced microbiological effects of the treatment if a more sensitive method had been used or if more heavily infected patients had been selected. The low TVC obtained in the beginning of the study confirms that the bacterial load was low at the start of the study. It is also possible that another sampling strategy should be used in case of sampling from failing implants. The tip of the paper point might harvest only a small fraction of bacterial flora of the peri-implantitis lesion, leading to a risk of false-negative samples compared with a selective culture method.

Another interesting finding is the gradual decrease of the target bacteria of the DNA–DNA hybridization values in the checkerboard method. A more immediate drop as seen for cultured *P. gingivalis* would have been expected; however, it is important to note that this distinction method records the presence of specific DNA and non-viable bacterial cells. It should be noted that 1% of TVC $10^5$ cells corresponds to 1000 cells, which is considerably lower than the detection level of the checkerboard technique. Both groups were treated with antimicrobial agents (Arestin® and chlorhexidine), both of which have a bacteriostatic/bactericidal effect on the target bacteria. If this exposure for antimicrobials is not followed by a mechanical cleaning or irrigation of the subgingival area, it may be so that the clearance period for the persisting DNA in the deep pocket is extended by several months.

Enteric rods and enterococci were included to study in order to analyse whether more resistant bacteria would increase in number because of the antimicrobial treatment and increase the risk for ''super-infection'' as noticed for certain cases of peri-implantitis in previous studies (Leonardt et al. 1999, 2003). A slight increase in the level of enteric rods could be noticed; however, the risk for super-infection did not seem to be important.

The use of adjunctive minocycline microspheres resulted in improvements in both probing depths and bleeding scores. The reductions of bleeding scores, although greater than for the chlorhexidine group, were modest. This may be related to the fact that the bleeding scores included bleeding assessed after microbial sampling. This sampling was made using four paper points placed at the deepest sites, which may have increased the potential to provoke bleeding at these sites. The use of microspheres containing minocycline has previously been found to be effective as an adjunct to mechanical treatment of periodontal (Williams et al. 2001, Henderson et al. 2002, Oringer et al. 2002, Van Dyke et al. 2002, Paquette et al. 2003) and peri-implantitis lesions (Heitz-Mayfield et al. 2003, Renvert et al. 2004). In the present study, the effectiveness could also be demonstrated for peri-implant lesions, as evidenced by improved results compared with the use of adjunctive chlorhexidine gel. The present study also indicates that clinical improvements obtained after use of minocycline microspheres may be sustained after a 12-month period. Nevertheless, the question to what extent the combined mechanical/minocycline treatment could be considered adequate for the treated lesion remains to be answered.

## A

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### References

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

Berglundh, T., Lindhe, J., Marinello, C., Ericsson, I. & Liljenberg, B. (1992) Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. *Clinical Oral Implants Research* **3**, 1–8.

Braß, M. & Anil, A. (1991) Periimplantäre Therapie mittels subgingivaler Irrigation und intraoperativem Einsatz von Salzstrahlgeräten. *Zeitschrift für Zahnärzliche Implantologie* **7**, 239–242.

Büchter, A., Meyere, U., Kruse-Löser, B., Joos, U. & Kleinheinz, J. (2004) Sustained release of doxycycline for the treatment of peri-implantitis: randomised controlled trial. *Brittish Journal of Oral and Maxillofacial Surgery* **42**, 439–444.

Ciancio, S. G., Lauciello, F., Shibly, O., Vitello, M. & Mather, M. (1995) The effect of an antiseptic mouthrinse on implant maintenance: plaque and peri-implant gingival tissues. *Journal of Periodontology* **66**, 962–965.

Flemmig, T. F. (1994) Infektionen bei osseointegrierten Implantaten - Hintergründe und klinische Implikationen. *Implantologie* **1**, 9–21.

Heitz-Mayfield, L., Haffajee, A. D. & Lang, N. P. (2003) Treatment of peri-implantitis using minocycline. *Journal of Clinical Periodontology* **30** (Suppl. 4), 10, (Abstract).

Henderson, R. J., Boyens, J. V., Holborrow, D. W. & Pack, A. R. C. (2002) Scaling and root-planing treatment with adjunctive subgingival minocycline. A clinical pilot study over six months, of sites adjacent to and remote from the antibiotic application. *Journal of the International Academy of Periodontology* **4/3**, 77–87.

Kao, R. T., Curtis, D. A. & Murray, P. A. (1997) Diagnosis and management of peri-implant disease. *Journal of California Dental Association* **12**, 872–880.

Klinge, B., Gustafsson, A. & Berglundh, T. (2002) A systematic review of the effect of anti-infective therapy in the treatment of peri-implantitis. *Journal of Clinical Periodontology* **29** (Suppl. 3), 213–225.

Lang, N. P., Mombelli, A., Tonetti, M. S., Brägger, U. & Hämmerle, C. H. F. (1997) Clinical trials on therapies for peri-implant infections. *Annals of Periodontology* **2**, 343–356.

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

Leonardt, Å., Dahlén, G. & Renvert, S. (2003) Five-year clinical, microbiological, and radiological outcome following treatment of peri-implantitis in man. *Journal of Periodontology* **74**, 1415–1422.

Mombelli, A. (2002) Microbiology and antimicrobial therapy of peri-implantitis. *Periodontology 2000* **28**, 177–189.

Mombelli, A. & Lang, N. P. (1998) The diagnosis and treatment of peri-implantitis. *Periodontology 2000* **17**, 63–76.

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.

Oringer, R. J., Van Dyke, T. E. & Lessem, J. (2002) The challenge of treating patients who smoke – the efficacy of Arestin®. *Journal of the International Academy of Periodontology* **4/3**, 89–94.

Papapanou, P. N., Madianos, P. N., Dahlén, G. & Sandros, J. (1997) ''Checkerboard'' versus culture: a comparison between two methods for identification of subgingival microbiota. *European Journal of Oral Science* **105**, 389–396.

Paquette, D., Minsk, L., Lessem, J. & Santucci, E. (2000) A pharmacokinetic study of a locally delivered minocycline therapeutic system (MPTC). *Journal of Clinical Periodontology* **27** (Suppl. 1), 24, (abstract).

Paquette, D., Oringer, R., Lessem, J., Offenbacher, S., Genco, R., Perrson, G. R., Santucci, E. A., & Williams, R. C. (2003) Locally delivered minocycline microspheres for the treatment of periodontitis in smokers. *Journal of Clinical Periodontology* **30**, 787–794.

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.

Porras, R., Anderson, G. B., Caffesse, R., Narendran, S. & Trejo, P. M. (2002) Clinical response to 2 different therapeutic regimens to treat peri-implant mucositis. *Journal of Periodontology* **73**, 1118–1125.

Renvert, S., Dahlén, G. & Wikström, M. (1996) Treatment of periodontal disease based on microbiological diagnosis. Relation between microbiological and clinical parameters dur-

ing 5 years. *Journal of Periodontology* **67**, 562–571.

Renvert, S., Lessem, J., Lindahl, C. & Svensson, M. (2004) Treatment of Incipient peri-implant infections using topical minocycline microspheres versus topical chlorhexidine gel as an adjunct to mechanical debridement. *Journal of the International Academy of Periodontology* **6**, 154–159.

Roos-JansÅker, A-M., Renvert, S. & Egelberg, J. (2003) Treatment of peri-implant infections: a literature review. *Journal of Clinical Periodontolog* **30**, 467–485.

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.

Schenk, G., Flemmig, T. F., Betz, T., Reuther, J. & Klaiber, B. (1997) Controlled local delivery of tetracycline HCl in the treatment of periimplant mucosal hyperplasia and mucositis. A controlled case series. *Clinical Oral Implants Research* **8**, 427–433.

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 Immunology* **19**, 352–362.

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

Van Dyke, T. E., Offenbacher, T., Braswell, M. & Lessem, J. (2002) Enhancing the value of scaling and root-planing: Arestin® clinical trial results. *Journal of the International Academy of Periodontology* **4/3**, 72–76.

Williams, R. C., Paquette, D. W., Offenbacher, S., Adams, D. F., Armitage, G. C., Bray, K., Caton, J., Cochran, D. L., Drisko, C. H., Fiorellini, J. P., Giannobile, W. V., Grossi, S., Guerrero, D. M., Johnson, G. K., Lamster, I. B., Magnsson, I., Oringer, R. J., Persson, G. R., Van Dyke, T. E., Wolff, L. F., Santucci, E. A., Rodda, B. E. and Lessem, J. (2001) Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. *Journal of Periodontology* **72**, 1535–1544.

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.

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**Clinical Relevance**

*Scientific rationale for the study:* Infections (peri-implantitis) do occur around dental implants. Peri-implantitis lesions are difficult to treat mechanically depending on the presence of threads and/or a rough surface that may retain the microflora.

*Principal findings:* This paper has demonstrated that the use of an adjunctive local antibiotic therapy (Arestin®) demonstrated improvements in probing depths that were sustained over 12 months.

*Practical implications:* It seems reasonable to combine mechanical treatment of peri-implantitis with local delivery of minocycline.