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A double-blind randomized clinical trial of subgingival minocycline for chronic periodontitis

José R. Cortelli[1)], Davi R. Aquino[1)], Sheila C. Cortelli[1)], Jonas Carvalho-Filho[2)], Caio V. G. Roman-Torres[1)] and Fernando O. Costa[3)]

[1)]Department of Dentistry, Periodontics Research Division, University of Taubaté, Taubaté, SP, Brazil
[2)]Institute of Basic Sciences, Molecular Biology Division, University of Taubaté, Taubaté, SP, Brazil
[3)]Department of Dentistry, Periodontics Research Division, Minas Gerais Federal University, Belo Horizonte, MG, Brazil

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Abstract: The purpose of this study was to evaluate the presence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *F. nucleatum* in 30 subjects with chronic periodontitis treated by scaling and root planing (SRP) plus minocycline (test group) during 12 months with regular trimester maintenance care. Additionally, we evaluated whether the beneficial effects of the therapy on the microbial flora persisted for 24 months. The test group (n = 15) and the control group [SRP plus placebo (n = 15)] were randomly assigned. After SRP, subjects received minocycline or placebo at the baseline, and at 3, 6, and 9 months at all sites with a periodontal pocket depth (PD) of ≥ 6 mm. Moreover, two homologous teeth, initially PD ≥ 6 mm, were clinically and microbially monitored by PCR at the baseline, and at 3, 6, 9, 12 and 24 months. Differences in mean PD values between groups were analyzed by Student's *t*-test ($P < 0.05$). The results for bacterial frequencies showed no significant differences between groups (Fisher's Exact test, $P < 0.05$) or between time-points (Friedman test, $P < 0.05$). We failed to detect any differences between groups related to the presence of target pathogens for 12 months. The effects of both therapies on the microbial flora did not persist for 24 months. The group without supportive periodontal therapy showed an improvement in the pattern of pathogens with either of the therapies. (J. Oral Sci. 50, 259-265, 2008)

Keywords: minocycline hydrochloride; bacteria; periodontitis; polymerase chain reaction.

Correspondence to Dr. José Roberto Cortelli, Rua Professor Nelson Freire Campelo, 343 Jardim Eulália, Taubaté, SP - Cep 12010-700 Brazil
Tel: +55-12-3631-2373
Fax: +55-12-3632-2947
E-mail: jrcortelli@uol.com.br

Introduction

It has been estimated that about 500 species of bacteria inhabit the human oral cavity (1,2,3). While the majority of these organisms are commensals, a subset are likely to be opportunistic pathogens that can cause systemic disease. Consequently, it is important to know that microorganisms are present in the oral cavity for the diagnosis and treatment of systemic as well as oral diseases. Recently (4), on the basis of the full 16S rRNA sequences for all cultivable and yet uncultivated species that colonize the oral cavity, over 600 species have been characterized.

Periodontal therapy is directed primarily towards reducing the number of pathogenic microorganisms in contact with periodontal tissues, because the role of microorganisms in the development and pathogenesis of periodontal disease is well established (5). It has been shown that scaling and root planing is an effective mechanical approach for reducing bacterial colonization to sub-threshold values and also for controlling the progression of periodontal disease. However, thorough subgingival scaling and root planing is technically demanding, as access to and visibility of the area are required, so that complete subgingival plaque and calculus removal is rarely achieved and sometimes ineffective (6), and may not eradicate species that can reach epithelial cells

and subepithelial connective tissues of the periodontium (7).

Microencapsulated minocycline hydrochloride in conjunction with scaling and root planing seems to provide significantly greater probing depth reduction than scaling and root planing alone (8,9). Previously, our group conducted a clinical and microbiological short-term study (from baseline to 120 days), to evaluate the presence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Campylobacter rectus*, *Eikenella corrodens* and *Fusobacterium nucleatum* and the clinical response to scaling and root planing associated with subgingival minocycline, in 36 subjects with chronic periodontitis (10). The results indicated that scaling and root planing plus subgingival minocycline was as effective as scaling and root planing plus placebo in reducing both the prevalence of target periodontal pathogens and probing depth. Later, we conducted another study focusing only on clinical data, but applying a longitudinal design (baseline, 3, 6, 9, 12 and 24 months). In the later investigation, the clinical response to scaling and root planing with the use of locally delivered minocycline microspheres was monitored in 26 individuals with chronic periodontitis (11). We observed that scaling and root planing plus minocycline achieved a higher reduction of periodontal pocket depth in 9 and 12 months than scaling and root planing plus placebo.

Supported by our previous results, we hypothesized whether the profile of longitudinal clinical response to scaling and root planing plus minocycline could be related to subgingival microorganisms. Thus, the first purpose of this double-blind randomized clinical and microbial trial was to evaluate the presence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *F. nucleatum* in subjects with chronic periodontitis treated with minocycline adjunctively to scaling and root planing during 12 months with trimester maintenance care visits. In addition, we had the opportunity to evaluate whether the beneficial effects of the test therapy on microbial flora persisted for 24 months.

Materials and Methods

Study design and clinical procedures

In 2002, a total of 59 subjects were recruited to participate in this double-blind randomized clinical and microbial study, all of whom were diagnosed as having advanced chronic periodontitis. However, 11 subjects left the study, and 18 did not comply with the study criteria; therefore, 30 subjects remained to the end of the study in 2005.

The study population received regular supportive periodontal therapy, at intervals of three months, for a total of 12 months. During this period, general dental treatment was offered to all participants. Thereafter, all the subjects were reminded about their periodontal appointments every trimester in the second year. However, since dental treatment had finished, some patients who had no dental pain or required no esthetic procedures considered that no further visits to the dental clinic were justified. After 24 months had passed, we were finally able to recruit only 30 subjects, aged 25 to 69 years (mean age 46.5 ± 12.2 years) out of the initial 59 who had received only supportive periodontal therapy during the first year. This unique condition offered us the opportunity to evaluate the study population with or without supportive periodontal maintenance care.

Data and personal information related to the medical and dental histories of the subjects were obtained by questionnaire. All subjects provided written informed consent, and the study was reviewed and approved by the institutional review board at the University of Taubaté, São Paulo, Brazil (protocol 013/02).

Subjects were excluded if they (1) had a chronic medical disease or condition; (2) required antibiotic premedication prior to dental treatment; (3) had taken antibiotics within 6 months prior to the clinical examination; or (4) had received periodontal therapy within the previous 6 months.

Periodontal and radiographic examinations were done to determine the periodontal status of all subjects, based on the criteria described by Armitage (12), including gingival redness, bleeding on probing, probing depth, and clinical attachment level measurements. During periodontal examination, one blinded, trained and calibrated examiner measured full-mouth periodontal probing depth (PD) at six sites per tooth, using a manual periodontal probe (PQWBR - Hu Friedy Mfg. Inc. Chicago, IL, USA). We analyzed the reproducibility of these measurements using kappa-statistics, and the examiner was considered calibrated when $k \geq 0.80$.

Each subject received local anesthesia followed by full-mouth scaling and root planing by a trained periodontist both supra- and subgingivally according to individual need. Before scaling and root planing (pre-experimental period), subgingival plaque samples were obtained from the selected sites (two non-molar homologous teeth presenting PD ≥ 6 mm associated with bleeding on probing and clinical attachment loss, without furcation involvement) in each participant to establish the microbiological baseline data.

At the end of scaling and root planing (baseline), two groups were randomly allocated; one was a test group (n = 15 subjects) that received locally delivered minocycline

(Arestin™, OraPharma, Inc., Warmister, PA, USA) in all sites PD ≥ 6 mm at this time and also at 3, 6, and 9 months, and the other was a control group (n = 15 subjects) that received placebo (polymer without minocycline - Byofórmula, Farmácia de Manipulação, São José dos Campos, SP, Brazil). Likewise, placebo polymer was applied at all sites PD ≥ 6 mm at the same time-points, i.e., at the baseline, 3, 6, and 9 months. Only one examiner assigned participants to the test or control group and the subjects were also blinded to minocycline or placebo treatment. In addition, a trained examiner evaluated plaque index (PI) (13) at the baseline and 3, 6, 9, 12 and 24 months.

The presence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *F. nucleatum* was determined in samples from the selected periodontal pockets of both the test and control groups at the baseline, and 3, 6, 9, 12 and 24 months.

Microbial sampling and laboratory procedures

Subgingival plaque samples were obtained using sterile paper points (Fine, Johnson and Johnson, New Brunswick, NJ, USA) inserted to the depth of the pockets after removal of supragingival plaque using a sterile curette. The paper points were removed after 10 seconds, and immediately placed in phosphate-buffered saline (pH 7.4) (Promega, Madison, WI, USA). The bacterial cells were dispersed by vortexing at maximal setting for 1 min and then maintained at -20°C until laboratory processing.

In the laboratory, the bacterial suspensions were thawed, centrifuged at 12,000 × *g* for 3 min, and the DNA was extracted from the pellet (Instagene purification matrix, BioRad Laboratories, Hercules, CA, USA). After centrifugation to remove cell debris, the supernatant was used for polymerase chain reaction (PCR) analysis. Briefly, 5 µl of the sample was added to 45 µl of reaction mixture containing 5 µl of 10 × PCR buffer (Promega, Madison, WI, USA), 1.25 unit of Taq DNA polymerase (Promega, Madison, WI, USA), and 0.2 mm of each deoxyribonucleotide (Pharmacia LKB, Piscataway, NJ, USA). PCR amplification was performed using a thermal cycler (Perkin Elmer Cetus, Wellesley, MA, USA) under standard conditions.

PCR products were analyzed using 1.5% agarose gel (Sigma, Dorset, UK) electrophoresis performed at 4 V/cm in Tris-acetate EDTA buffer (Promega, Madison, WI, USA). The gel was stained with 0.5 µg/ml ethidium bromide (Amershan, Airlington Heights, USA) and photographed under 300 nm ultraviolet light.

Statistical analysis

Mean values of periodontal probing depth and PI scores between the test and the control groups were analyzed statistically by Student's *t* test. Differences were considered statistically significant at $P < 0.05$. The association of frequencies of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *F. nucleatum* between the test and the control groups were analyzed using Fisher's exact test. Moreover, bacterial data analysis included comparisons between groups by Mann-Whitney test and also between time-points determined by Friedman test. All analyses were performed using Statistical Package for Social Sciences release 12.0 (SPSS Inc. Chicago, USA). Results were considered significant at $P < 0.05$.

Results

Table 1 shows data related to the profile of periodontal probing depth (PD) from the baseline until 24 months. At the baseline the mean PD values for the test and control groups did not differ. When PD values were compared, there was a bigger reduction in the test group in the 9th ($P = 0.0188$) and in the 12th ($P = 0.0470$) months, however, no significant differences were observed at 3, 6 and 24 months. When we considered the mean PD values longitudinally, in the test group we observed significant reductions of PD from the baseline (A) to 3 months (C), from 6 (CD) to 9

Table 1 Mean PD values (in mm) comparing control and test groups in each time (expressed by *P* values) and compared longitudinally (from baseline to 24 months)

Time-points	Control Group (Mean ± SD)	Test Group (Mean ± SD)	*P* Value (*P* <0.05)
Baseline	7.73 ± 0.88 A	7.47 ± 0.74 A	0.6457
3 Months	5.00 ± 1.51 C	4.30 ± 1.05 C	0.2101
6 Months	4.97 ± 1.62 C	4.07 ± 1.16 CD	0.1901
9 Months	5.10 ± 1.49 C	3.97 ± 1.01 D	0.0188*
12 Months	5.17 ± 1.55 C	3.80 ± 0.80 D	0.0470*
24 Months	5.90 ± 2.20 B	5.23 ± 1.40 B	0.9100

*Means significant differences between test and control groups; different capital letters represent significant differences among time-points (from baseline to 24 months) within each column. Student's *t*-test ($P < 0.05$)

and 12 (D) months, and a significant increase of PD in the 24th (B) month. In the control group we also observed significant reductions of PD from the baseline (A) to 3 months (C), no significant difference from 3 (C) until 12 months (C), and a significant increase of PD in the 24th (B) month.

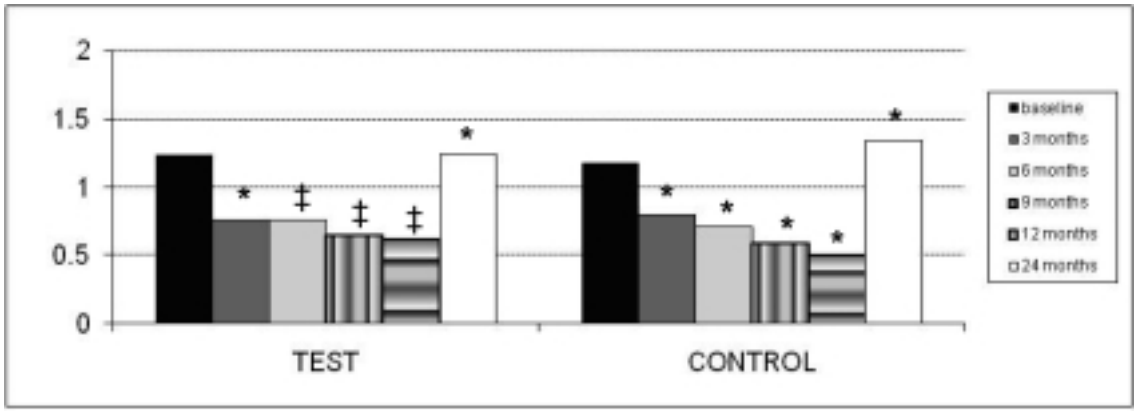

Fig. 1 Comparison of mean Plaque Index values between the control and test groups at the baseline and 3, 6, 9, 12 and 24 months.
* Statistically significant difference ($P < 0.05$); ‡ No statistically significant difference

Table 2 Frequencies of bacteria in test and control groups at baseline, 3, 6, 9, 12, and 24 months

Time-points	Bacteria	Groups				Fisher's exact test P^*
		Control		Test		
		Absent	Present	Absent	Present	
Baseline	*A. actinomycetemcomitans*	12	3	9	6	0.2678
	P. gingivalis	9	6	6	9	0.4661
	P. intermedia	12	3	9	6	0.2678
	E. corrodens	13	2	13	2	1.0
	F. nucleatum	10	5	10	5	1
3 months	*A. actinomycetemcomitans*	13	2	11	4	0.6513
	P. gingivalis	11	4	9	6	0.4725
	P. intermedia	10	5	14	1	0.1686
	E. corrodens	10	5	13	2	0.3898
	F. nucleatum	12	3	13	2	1.0
6 months	*A. actinomycetemcomitans*	15	0	14	1	1.0
	P. gingivalis	13	2	14	1	1.0
	P. intermedia	14	1	14	1	1.0
	E. corrodens	12	3	12	3	1.0
	F. nucleatum	12	3	12	3	1.0
9 months	*A. actinomycetemcomitans*	15	0	14	1	1.0
	P. gingivalis	13	2	28	2	0.4828
	P. intermedia	15	0	15	0	-
	E. corrodens	10	5	10	5	1.0
	F. nucleatum	11	4	12	3	1.0
12 months	*A. actinomycetemcomitans*	15	0	14	1	1.0
	P. gingivalis	13	2	15	0	0.4828
	P. intermedia	15	0	15	0	-
	E. corrodens	10	5	10	5	1.0
	F. nucleatum	11	4	12	3	1.0
24 months	*A. actinomycetemcomitans*	14	1	14	1	1.0
	P. gingivalis	12	3	10	5	0.4482
	P. intermedia	15	0	14	1	1.0
	E. corrodens	7	8	9	6	0.7152
	F. nucleatum	0	7	10	5	0.7104

* probability values

The PI showed a statistically significant reduction in the test group ($P < 0.05$) from the baseline to 3 months. No statistically significant difference was observed in PI between 3 and 12 months; however, in the 24th month, a significant increase was observed. In the control group, a statistically significant reduction was observed at the baseline and at 3, 6, 9 and 12 months, followed by an increase in the 24th month (Fig. 1).

With regard to frequencies of bacteria (Table 2), the results showed no significant differences either between groups or between time-points (Table 3) from the baseline to 24 months (Mann-Whitney test).

In the control group, differences among time-points related to *P. gingivalis* were not observed. Similar results were found for *E. corrodens* in the test group and also for *F. nucleatum* in both groups. Mean values for *A. actinomycetemcomitans* and *P. intermedia* decreased from 6 months onwards. For *P. gingivalis* in the test group and *E. corrodens* in the control group, mean values started to decrease earlier, from 3 months until 12 months. However, in the 24th month these values increased, becoming similar to those reached in the 3rd month.

Discussion

Since bacteria represent the most important etiologic factor for periodontitis, the purpose of periodontal treatment is to suppress or eliminate target species from supra- and subgingival areas, allowing recolonization by beneficial bacterial species. Thus, the anti-infection approach in the active phase of periodontal treatment consists of a number of measures to reduce the total bacterial load. Deep periodontal pockets that remain after full-mouth scaling and root planing and oral hygiene instructions are treated by surgery or other treatment strategies, including the use of systemic and/or local antibiotic therapy. Additionally, the frequency of maintenance care is an essential factor for long-term success of periodontal therapies, depending on a patients' susceptibility to periodontal disease. Intervals ranging from two weeks to six months have been used by clinicians (14).

Taking anti-infective periodontal therapy into consideration, the first purpose of this study was to investigate whether scaling and root planing accompanied by subgingival use of minocycline could result in improved microbial outcomes. In order to do this, we monitored data related to the frequencies of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *E. corrodens* and *F. nucleatum* during 12 months. In addition we re-evaluated the occurrence of these pathogens after 1 year without supportive periodontal therapy. The decision to use a local drug delivery system in combination with scaling and root planing was supported by evidence from the dental literature (5,8,9,10,11,15-17), indicating that as probing depth increases, scaling and root planing alone becomes less efficient.

The initiation and progression of periodontal disease is attributed to the presence of elevated counts of pathogenic bacteria within the gingival crevice. Likewise, periodontal pathogens such as *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, *Treponema denticola* and *E. corrodens* have been associated with chronic periodontitis (18). Many researchers agree that the success of periodontal therapy depends on the reduction of these microorganisms in subgingival areas.

In the present study, *P. gingivalis* was the most prevalent bacterium at the baseline in both the test and the control groups. This finding is in agreement with other studies showing that *P. gingivalis* is one of the most important pathogens in advanced periodontitis based on its high prevalence in progressive periodontitis lesions (19,20), and that this organism should be considered a key pathogen in destructive periodontal disease. Indeed, previous data from Brazilian and Chilean cohorts showed that *P. gingivalis* was present in a higher proportion of periodontal pockets

Table 3 Mean bacteria values according to time-points in test and control groups

Bacteria	Groups	Time-points / months					
		baseline	3	6	9	12	24
A. a	Control	3.90 a	3.70 a	3.30 b	3.30 b	3.30 b	3.50 ab
	Test	4.23 a	3.83 ab	3.23 b	3.23 b	3.23 b	3.23 b
P.g	Control	4.07 a	3.67 a	3.27 a	3.27 a	3.27 a	3.47 a
	Test	4.60 a	4.00 ab	3.00 cd	2.80 d	2.80 d	3.80 b
P.i	Control	3.80 ab	4.20 a	3.40 b	3.20 bc	3.20 bc	3.20 bc
	Test	4.40 a	3.40 bc	3.40 bc	3.20 c	3.20 c	3.40 bc
E.c	Control	2.97 b	3.57 ab	3.17 b	3.57 ab	3.57 ab	4.17 a
	Test	3.13 a	3.13 a	3.33 a	3.73 a	3.73 a	3.93 a
F.n	Control	3.63 a	3.23 a	3.23 a	3.43 a	3.43 a	4.03 a
	Test	3.80 a	3.20 a	3.40 a	3.40 a	3.40 a	3.80 a

Different lower-case letters mean significant differences among time-points within each line (Friedman test, $P < 0.05$).

in groups with advanced chronic periodontitis (21).

Another finding verified in our study was that in the 9th, 12th and 24th months, the most prevalent pathogen in the test and control groups was *E. corrodens*. Ebersole et al. (22) reported that *E. corrodens* was associated with sites in periodontal pockets, whereas another study (23) demonstrated a weak association with chronic periodontitis, and a third study (24) showed that there was no significant difference between active and inactive sites in the distribution of *E. corrodens*. In 2002, Avila-Campos and Velasques-Mendez (25) evaluated the prevalence of putative periodontopathogens in periodontal patients and healthy subjects and found that *E. corrodens* and *F. nucleatum* were not significantly associated with periodontal disease. Our results are in contrast with their findings because we found high prevalence of *E. corrodens* at both baseline and post-therapy, despite the lack of maintenance care. This contrast is especially reinforced by findings related to our control group due to the observed increasing in *E. corrodens* prevalence besides PD increasing.

In 1993, a study (15) reported that concentrations of *P. gingivalis* and *P. intermedia* were lower after minocycline ointment treatment than after scaling and root planing in combination with placebo ointment at 2, 4, 6, and 12 weeks post-treatment, as well as the concentration of *A. actinomycetemcomitans* at 6 and 9 weeks. Another study (16) demonstrated a significant reduction in the prevalence of periodontal pathogens over a 15-month period, but no significant reduction was observed when local delivery of minocycline was used. Three years later, a further study (17) demonstrated significant differences in outcome for several microbial species including *P. gingivalis*, *P. intermedia*, *C. rectus*, *T. denticola*, *E. corrodens*, *F. nucleatum*, and *A. actinomycetemcomitans* at various follow-up points from month 1 to month 15 between patients receiving adjunctive minocycline ointment and nonsurgical scaling and root planing alone. There were significantly greater reductions only in the *P. gingivalis*, *T. denticola*, and *C. rectus* counts from the baseline, but for the other microbial species the changes were not significant. In general, both therapies allowed for benefits to the microbial flora. However, in the present study when we compared the presence of all target pathogens between the two groups, we clearly observed no statistically significant difference after comparisons from the baseline to 24 months, i.e., with or without trimester supportive periodontal therapy.

Haffajee et al. (26) reported that there appears to be a consensus that treatment of the infectious component of periodontal diseases achieves its maximum benefit from 3 to 6 months after therapy, and that any clinical and microbiological benefits slowly reverse thereafter. Although between-group differences were not reported, the microbial reductions during the first year did not persist for 24 months.

In our study, except for *A. actinomycetemcomitans* (test group) and *P. intermedia* (control group), all bacterial species increased without supportive periodontal therapy (Table 2). Probably these microbial profiles are related to the observed increase in mean PD values (Table 1). Our results confirm that lack of supportive periodontal therapy results in a worsening of microbial and PD profiles. When patients were asked why they failed to comply with 12-month maintenance care, two principal reasons were cited: the first was absence of pain related to periodontal disease, and the other was related to financial costs mainly associated with transport.

In conclusion, the data from this double-blind randomized clinical and microbial trial failed to demonstrate any difference between scaling and root planing, with or without subgingival minocycline treatment, in terms of the presence of target periodontal pathogens within 12 months. The effects on microbial flora achieved by both therapies did not persist for 24 months. Absence of supportive periodontal therapy increased the subgingival presence of pathogens with either of the therapies.

References

1. Moore WE, Moore LV (1994) The bacteria of periodontal diseases. Periodontol 2000 5, 66-77
2. Socransky SS, Haffajee AD (1994) Evidence of bacterial etiology: a historical perspective. Periodontol 2000 5, 7-25
3. Kolenbrander PE, Palmer RJ Jr, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI (2006) Bacterial interactions and successions during plaque development. Periodontol 2000 42, 47-79
4. Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, Paster BJ (2003) Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J Clin Microbiol 41, 558-563
5. Petersilka GJ, Ehmke B, Flemming TF (2002) Antimicrobial effects of mechanical debridement. Periodontol 2000 28, 56-71
6. Rateitschak-Plüss EM, Schwarz JP, Guggenheim R, Düggelin M, Rateitschak KH (1992) Non-surgical periodontal treatment: where are the limits? An SEM study. J Clin Periodontol 19, 240-244
7. Adriaens PA, De Boever JA, Loesche WJ (1988) Bacterial invasion in root cementum and radicular dentin of periodontally diseased teeth in humans.

A reservoir periodontopathic bacteria. J Periodontol 59, 222-230
8. Williams RC, Paquette DW, Offenbacher S, Adams DF, Armitage GC, Bray K, Caton J, Cochran DL, Drisko CH, Fiorellini JP, Giannobile WV, Grossi S, Guerrero DM, Johnson GK, Lamster IB, Magnusson I, Oringer RJ, Persson GR, Van Dyke TE, Wolff LF, Santucci EA, Rodda BE, Lessem J (2001) Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. J Periodontol 72, 1535-1544
9. Lessem J, Hanlon A (2004) A post-marketing study of 2805 patients treated for periodontal disease with Arestin. J Int Acad Peridontol 6, Suppl 4, 150-153
10. Querido SMR, Cortelli SC, Araújo MWB, Cortelli JR (2004) Clinical and microbial evaluation of dental scaling associated with subgingival minocycline in chronic periodontitis subjects. Braz Oral Res 18, 110-115
11. Cortelli JR, Querido SMR, Aquino DR, Ricardo LH, Pallos D (2006) Longitudinal clinical evaluation of adjunct minocycline in treatment of chronic periodontitis. J Periodontol 77, 161-166
12. Armitage GC; Research, Science and Therapy Committee of the American Academy of Periodontology (2003) Diagnosis of periodontal disease. J Periodontol 74, 1237-1247
13. Silness J, Loe H (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22, 121-135
14. Wilson TG Jr (1996) Supportive periodontal treatment introduction – definition, extent of need, therapeutic objectives, frequency and efficacy. Periodontol 2000 12, 11-15
15. van Steenberghe D, Bercy P, Kohl J, De Boever J, Adriaens P, Vanderfaeillie A, Adriaenssen C, Rompen E, De Vree H, McCarthy EF, Vandenhoven G (1993) Subgingival minocycline hydrochloride ointment in moderate to severe chronic adult periodontitis: a randomized, double-blind, vehicle-controlled, multicenter study. J Periodontol 64, 637-644
16. Timmerman MF, van der Weijden GA, van Steenbergen TJ, Mantel MS, de Graaff J, van der Velden U (1996) Evaluation of the long-term efficacy and safety of locally-applied minocycline in adult periodontitis patients. J Clin Periodontol 23, 707-716
17. van Steenberghe D, Rosling B, Soder PO, Landry RG, van der Velden U, Timmerman MF, McCarthy EF, Vandenhoven G, Wouters C, Wilson M, Matthews J, Newman HN (1999) A 15-month evaluation of the effects of repeated subgingival minocycline in chronic adult periodontitis. J Periodontol 70, 657-667
18. Noiri Y, Li L, Ebisu S (2001) The localization of periodontal disease-associated bacteria in human periodontal pockets. J Dent Res 80, 1930-1934
19. Slots J, Bragd L, Wikström M, Dahlén G (1986) The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* in destructive periodontal disease in adults. J Clin Periodontol 13, 570-577
20. Slots J, Listgarten MA (1988) *Bacteroides gingivalis*, *Bacteroides intermedius* and *Actinobacillus actinomycetemcomitans* in human periodontal diseases. J Clin Periodontol 15, 85-93
21. Cortelli JR, Cortelli SC, Jordan S, Haraszthy VI, Zambon JJ (2005) Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol 32, 860-866
22. Ebersole JL, Taubman MA, Smith DJ, Frey DE, Haffajee AD, Socransky SS (1987) Human serum antibody responses to oral microorganisms. IV. Correlation with homologous infection. Oral Microbiol Immunol 2, 53-59
23. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25, 134-144
24. Dzink JL, Tanner AC, Haffajee AD, Socransky SS (1985) Gram negative species associated with active destructive periodontal lesions. J Clin Periodontol 12, 648-659
25. Avila-Campos MJ, Velásquez-Meléndez G (2002) Prevalence of putative periodontopathogens from periodontal patients and healthy subjects in São Paulo, SP, Brazil. Rev Inst Med trop S Paulo 44, 1-5
26. Haffajee AD, Teles RP, Socransky SS (2006) The effect of periodontal therapy on the composition of the subgingival microbiota. Periodontol 2000 42, 219-258