# Association of Antimicrobial and Clinical Efficacy: Periodontitis Therapy with Minocycline Microspheres

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**Abstract**

**Objective:** The objective of this study was to investigate the association between the antimicrobial and clinical efficacy of minocycline hydrochloride microspheres when used adjunctively with scaling and root planing. **Methods:** 127 subjects with moderate-to-advanced chronic periodontitis were randomly assigned to receive either minocycline microspheres plus scaling and root planing (n = 62) or scaling and root planing alone (n = 65). Deoxyribose nucleic acid analysis and clinical data were obtained at baseline and 30 days after treatment. End points included changes in the mean sum of red complex bacteria, pocket depth, number of deep pockets, bleeding on probing, and clinical attachment level from baseline to day 30. Regression analysis determined the association between microbiological and clinical efficacy. **Results:** Minocycline microspheres plus scaling and root planing reduced pocket depth, the number of deep pockets and bleeding on probing, and increased clinical attachment level significantly more than scaling and root planing alone ($p < 0.05$). Comparing minocycline microspheres plus scaling and root planing with scaling and root planing alone, the number needed to treat for a 2 mm pocket depth reduction difference was 6.5. Pocket depth reduction correlated significantly with a decrease in the numbers and proportions of red complex bacteria. Minocycline microspheres significantly improved all clinical parameters compared to scaling and root planing alone. **Conclusions:** The addition of minocycline microspheres to scaling and root planing led to a greater reduction in the proportions and numbers of red complex bacteria. The reduction in pocket depth was significantly correlated with the reduction of the proportions and numbers of red complex bacteria. Additionally, there were statistically greater improvements in all clinical parameters examined.

*Key words: Minocycline; periodontitis; pocket depth; scaling and root planing*

## Introduction

Periodontitis is primarily caused by an inflammatory response to infection with Gram-negative anaerobic bacteria (Socransky and Haffajee, 2005). Logic suggests that effective therapy in the successful treatment of this disease should be aimed at reducing or eliminating putative periodontal bacteria (Teles *et al.*, 2006). Scaling and root planing (SRP) is considered the gold standard procedure for stabilizing the attachment level in a patient population with mild-to-moderate periodontitis (Haffajee *et al.*, 1997). Mechanical manipulation of the subgingival environment with SRP induces considerable changes in the composition and relationships among the microbial inhabitants of the periodontal pocket (Haffajee *et al.*, 2006). SRP has been shown to significantly reduce the bacterial load, which results in improvements in all clinical parameters of periodontitis, including pocket depth (PD), clinical attachment level (CAL), and gingival inflammation (Isidor and Karring, 1986; Lindhe *et al.*, 1984; Pihlstrom

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*et al.*, 1983; Ramfjord *et al.*, 1987). Additionally, a positive effect has been demonstrated when the prevalence of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythus* (currently *T. forsythia*) has decreased following SRP (Haffajee *et al.*, 1997). However, the response to SRP is not universal because some sites continue to lose periodontal attachment during the maintenance phase of therapy (Pihlstrom *et al.*, 1983). This loss may be attributed to pathogenic bacteria that have survived mechanical therapy or to re-infection of the treated sites (Shiloah *et al.*, 1997).

Systemic administration of antimicrobials has been demonstrated to improve the results obtained by SRP and other forms of periodontal therapy in patients with chronic and aggressive periodontitis (Haffajee *et al.*, 2003). Nevertheless, which antimicrobial to use and at what dosage and duration remains inconclusive. Indiscriminate use of systemic antibiotics can interfere with normal body flora and have the potential to cause significant adverse reactions (Kornman and Karl, 1982). Additionally, patient compliance and cooperation with the prescribed regimen is another major obstacle to systemic therapy. The local application of antimicrobial agents overcomes many of these problems. They offer a local, site-specific approach and can be administered at a high, sustainable concentration. This helps to avoid the possible negative effects of systemic administration. Also, they are less dependent on patient compliance. Their effectiveness as an adjunctive therapy in the treatment of chronic periodontitis has been well documented (Bonito *et al.*, 2005; Goodson *et al.*, 1979; Hanes and Purvis, 2003; Meinberg *et al.*, 2002; Newman *et al.*, 1994; Soskolne *et al.*, 1997; Wennstrom *et al.*, 2001; Williams *et al.*, 2001). Their effects on the subgingival microflora, however, have not been well established.

Minocycline hydrochloride 1 mg microspheres (MM) are locally applied, sustained-release antimicrobials that were developed to augment the effectiveness of SRP. They have been demonstrated to statistically decrease probing depths and to prevent bone loss (Meinberg *et al.*, 2002; Williams *et al.*, 2001). The purpose of this article is to examine the association between the antimicrobial and clinical effects (defined by changes in PD, numbers of deep pockets [PD ≥ 5 mm], BOP [bleeding on probing], and CAL) of MM when used as an adjunctive therapy to SRP.

## Materials and methods

### *Recruitment*

One hundred thirty subjects from 5 periodontology clinics in the United States were voluntarily enrolled beginning on January 21, 2004. All were required to provide written informed consent prior to enrollment. Inclusion criteria involved subjects between the ages of 30 and 65 years who were in good general health and had at least 16 teeth (excluding third molars and implants) and at least five sites with PD greater than or equal to 5 mm (test sites) in nonadjacent interproximal spaces. Exclusion criteria included serious systemic disease that could influence the course of periodontal disease (e.g., diabetes, autoimmune diseases), pregnancy or lactation, not using acceptable methods of birth control by females of child-bearing potential, any periodontal therapy within the previous three months (excluding maintenance therapy), systemic or local antimicrobial therapy within the previous three months, a requirement for prophylactic antimicrobials, aggressive periodontitis, necrotizing ulcerative gingivitis, gross dental decay or tetracycline allergy. Approval was obtained from all relevant institutional review boards and all subjects gave informed consent prior to the study.

### *Study design and methods*

This was a multicenter, single-blind, randomized, parallel-group, phase IV study. To ensure adequate blinding, the examiner was blinded to the study group, and a separate clinician administered all treatments. Prior to the study, examiners were calibrated for probing technique and CAL evaluation, and a single "gold standard" study coordinator calibrated each of the five centers on plaque sampling and processing. To standardize assessments, a single laboratory (The Forsyth Institute, Boston, MA) performed all microbiological analyses. All enrolled subjects received a full-mouth examination at baseline to measure PD, the number of deep pockets, BOP, and CAL. Subgingival plaque samples from five test sites were collected using a sterile curette and sent for microbiological (DNA probe) analysis. This analysis has been previously described by Socransky and colleagues (2004). After the baseline evaluation, subjects were assigned by pairwise randomization to one of two treatment groups. One group received MM + SRP and the other group received SRP alone. A periodontist, dentist, or registered dental hygienist affiliated with the study center performed the treatment. SRP was to be completed within a maximum of two visits no more than 10 days apart. Subjects randomized to the MM + SRP group received a single unit dose of MM (1 mg of minocycline HCl in a vehicle of approximately 3 mg of polyglycolide-co-dl-lactide or PGLA) in each periodontal pocket greater than or equal to 5 mm in depth. Subjects were instructed to postpone brushing their teeth for 12 hours and to abstain from using interdental cleaning devices (e.g., floss) for 10 days following administration of MM. Clinical measurements and the collection of subgingival plaque samples from the test sites were repeated on day 30.

### End points

The primary end points of the study were changes in the mean sum of red complex bacteria (RCB) proportions (i.e., *P. gingivalis* % + *T. forsythia* % + *T. denticola* % [averaged for each subject]) and the mean sum of RCB numbers (N x $10^5$) relative to the 40 periodontal bacteria analyzed from baseline to day 30. Secondary end points included changes in the total proportions and total numbers of each RCB relative to the 40 periodontal bacteria measured, as well as mean changes in PD, number of deep pockets, BOP, and CAL. The results reported herein evaluate the association between changes in the mean proportion and number of RCB (sum and individual) and mean changes in PD, BOP, and CAL from baseline to day 30. Safety assessments included reported, elicited and observed adverse events throughout the study, as well as the intraoral examination of soft and hard tissues at baseline and at day 30.

### Statistical analysis

Discrete baseline demographic differences between treatment groups were evaluated using Pearson Chi-square. Differences in continuous variables (i.e., age, microbiologic, and clinical measures) were evaluated using 1-way analysis of variance (ANOVA). Outcome measures were evaluated for underlying distributional characteristics, and $\log_{10}$ transformations were performed as necessary to normalize the data. ANOVA and ANCOVA (analysis of covariance) were used to evaluate mean differences in measures between treatment groups from baseline to day 30. Regression analysis using microbiological parameters as independent variables was used to determine the association between changes in microbiological end points and reduction in PD. Statistical significance was determined by a $p < 0.05$. Goodson and colleagues (2007) have published a more detailed description of the statistical analyses performed in this study.

## Results

One hundred thirty subjects were enrolled into the study and randomly assigned to receive MM + SRP or SRP alone. Of these, three subjects were excluded from the microbiologic and clinical efficacy analysis: two subjects because of periodontal abscess formation (both in the MM + SRP group) and one subject because subgingival plaque samples were not obtained at day 30 (SRP group). A total of 127 subjects were included in the efficacy analysis: 62 in the MM + SRP group and 65 in the SRP alone group. The demographic distribution of subjects included in the two treatment groups was well matched at baseline. In the MM + SRP group, the mean age was 50.9 years, 39% were female, and 63% were Caucasian. Corresponding values in the SRP alone group were 48.9 years, 54% female, and 60% Caucasian. Baseline microbiological and clinical variables (e.g., PD, BOP, CAL) were similar in both groups. The mean dose exposure of the 62 subjects treated with MM was 24.0 (± 12.7) mg (range, 5 to 72 mg), based on a dosage of 1 mg per treated site. The last subject completed the trial on August 12, 2004.

### Microbiologic end points

At 30 days, subjects treated with MM + SRP achieved a significantly greater mean reduction in the proportion of RCB than those treated with SRP alone (6.5% vs 5.0%; $p = 0.0005$). MM + SRP also reduced the mean number of RCB at day 30 to a significantly greater extent than did SRP alone. Mean RCB numbers were reduced 50% (from 18.9 x $10^5$ to 9.5 x $10^5$) by MM + SRP, and 26% (from 19.3 x $10^5$ to 14.3 x $10^5$) by SRP alone ($p = 0.002$). MM + SRP also reduced the proportions and numbers of each of the RCB individually relative to the 40 bacteria measured to a greater extent than SRP alone (*Table 1*). These reductions were all statistically significant ($p < 0.05$), with the exception of the reduction in the number of *T. forsythia* ($p = 0.07$). Additional microbiological results have been published in detail elsewhere (Goodson *et al.*, 2007; Grossi *et al.*, 2007).

### Clinical end points

All clinical outcomes are reported for sites initially deep at baseline (i.e., PD ≥ 5 mm). MM + SRP significantly improved all clinical outcome measures compared with SRP alone (*Table 2*). Mean reductions in PD of deep sites were 1.38 mm in the MM + SRP group compared with 1.01 mm in the SRP alone group ($p = 0.00004$). MM + SRP reduced the mean number of deep pockets by 51.6%, from 24.0 to 11.6, whereas SRP alone reduced the number by only 37.3%, from 28.4 to 17.8. Mean reduction in BOP in sites initially deep at baseline was 25.2% in the MM + SRP group compared with only 13.8% in the SRP alone group, nearly a 2-fold difference ($p = 0.009$). Finally, MM + SRP significantly increased CAL, the principal indicator of periodontal stability, compared with SRP alone (1.16 mm vs 0.80 mm, respectively; $p = 0.0004$). Similarly, the percentage of initially deep sites with increase in CAL by greater than or equal to 2 mm at 30 days was statistically significant ($p = 0.002$).

### Association between microbiological and clinical end points

A decrease in the numbers and proportions of RCB was associated with a significant ($p \leq 0.001$) decrease in PD (*Table 3*). Regression analysis between PD reduction and RCB proportions resulted in a linear association described by the following equation: PD reduction (mm) = -0.027 x RCB (%) + 0.71. The relatively high level of R in this analysis suggests that this equation

**Table 1.** Mean reduction of the proportions and numbers of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in subjects treated with minocycline HCl microspheres (MM) plus scaling and root planing (SRP) or SRP alone.

| Parameter | MM + SRP | SRP alone | *p value* |
|---|---|---|---|
| *Porphyromonas gingivalis* proportions | 2.5% | 1.7% | 0.00007 |
| *Tannerella forsythia* proportions | 2.7% | 2.5% | 0.0009 |
| *Treponema denticola* proportions | 1.4% | 0.8% | 0.004 |
| *Porphyromonas gingivalis* numbers | $3.71 \times 10^5$ | $1.54 \times 10^5$ | 0.0001 |
| *Tannerella forsythia* numbers | $4.32 \times 10^5$ | $3.57 \times 10^5$ | 0.07 |
| *Treponema denticola* numbers | $1.40 \times 10^5$ | $-0.003 \times 10^{5*}$ | 0.01 |

*Negative values indicate an increase from baseline to 30 days.

**Table 2.** Summary of clinical measurements: reduction from baseline at day 30.

| Clinical end point | MM + SRP | SRP alone | *p value* |
|---|---|---|---|
| PD reduction (mm) | 1.38 | 1.01 | 0.00004 |
| Reduction in number of deep pockets | 12.4 | 10.6 | 0.01 |
| % of sites with ≥5 mm PD at baseline – PD reduced by ≥ 2 mm | 46.6% | 31.2% | 0.001 |
| % of sites with ≥ 5 mm PD at baseline - CAL increased by ≥ 2 mm | 40.6% | 28.6% | 0.002 |
| Bleeding on probing reduction (%) | 25.2 | 13.8 | 0.009 |
| CAL gain (mm) | 1.16 | 0.80 | 0.0004 |

CAL, clinical attachment level; MM, minocycline HCl microspheres; PD, pocket depth; SRP, scaling and root planing.

**Table 3.** Association between pocket depth reduction and red complex bacteria at 30 days computed by regression analysis of data from both treatment groups.

| Effect on red complex bacteria | *F-ratio | *p value* | r |
|---|---|---|---|
| Bacterial numbers | 10.56815 | 0.00149 | 0.600 |
| Bacterial proportions | 11.19368 | <0.000001 | 0.614 |

*F-ratio is the variance ratio between the variables being tested, *p* value is the statistical significance level of this ratio and r is the correlation coefficient.

**Table 4.** Correlation coefficients (Pearson) between clinical end points and the proportion of each red complex bacteria at 30 days.

| Red complex bacteria | PD reduction | Reduction in BOP | CAL gain |
|---|---|---|---|
| *Porphyromonas gingivalis* | -0.201 | -0.236 | -0.209 |
| *Tannerella forsythia* | -0.352 | -0.249 | -0.291 |
| *Treponema denticola* | -0.224 | -0.204 | -0.142 |

BOP, bleeding on probing; CAL, clinical attachment level; PD, pocket depth.

describes approximately 38% of the variability between PD reduction and RCB (%). Unlike PD, changes in the RCB proportion or number were not significantly associated with changes in other clinical end points (i.e., number of deep pockets, BOP, or CAL; data not shown). Nevertheless, changes in each of the three RCB correlated with changes in PD, BOP, and CAL (*Table 4*). The strongest correlation was found between *T. forsythia* and reduction of PD (r = -0.352), followed by *T. denticola* (r = -0.224) and *P. gingivalis* (r = -0.201). Although these correlations are weak, they are of the magnitude commonly cited for single bacteria. As demonstrated in *Table 3*, correlations with total RCB proportions (0.614) and RCB numbers (0.600) were considerably greater. The association between PD reduction and the proportion of each of the three RCB at day 30 is illustrated in *Figures 1, 2*, and *3*. These figures show that greater clinical improvement (i.e., PD reduction) was associated with lower proportions of each of the RCB. Similar to PD reduction, regression analysis revealed that the correlation between CAL and changes in RCB proportions could be described by the following equation: CAL changes (mm) = -0.035 x RCB (%) + 1.28.

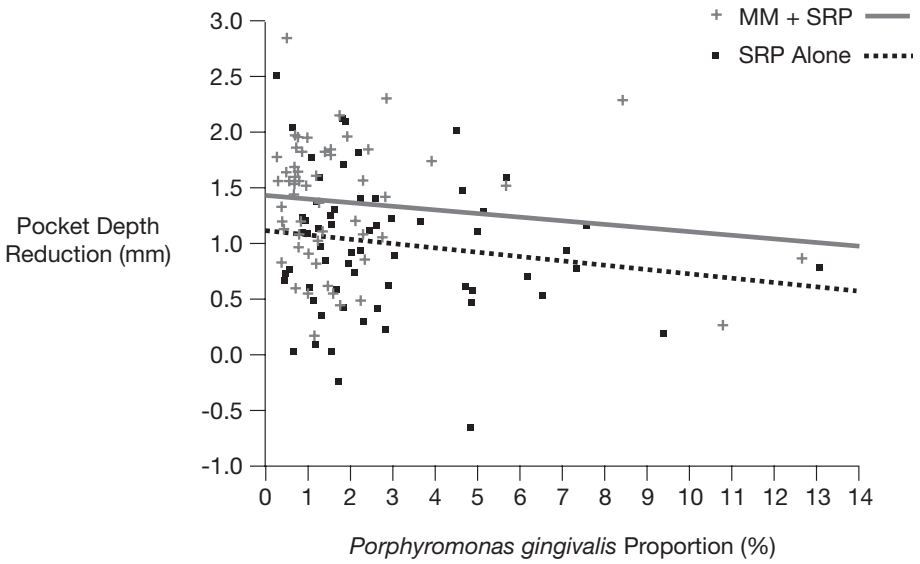


***Figure 1. Pocket depth reduction and Porphyromonas gingivalis proportions at 30 days for each treatment. MM, minocycline HCl microspheres; SRP,scaling and root planing.***

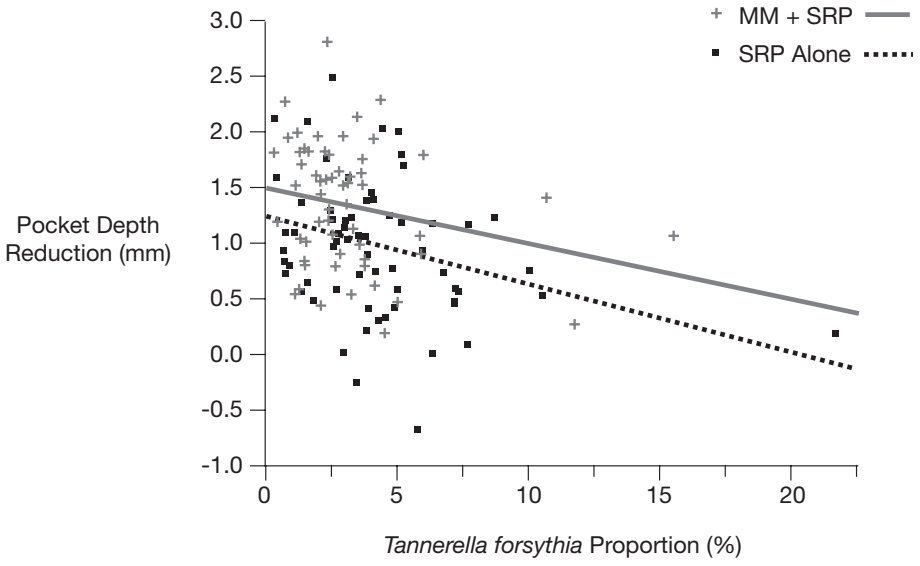


***Figure 2. Pocket depth reduction and Tannerella forsythia proportions at 30 days for each treatment. MM,minocycline HCl microspheres; SRP, scaling and root planing.***

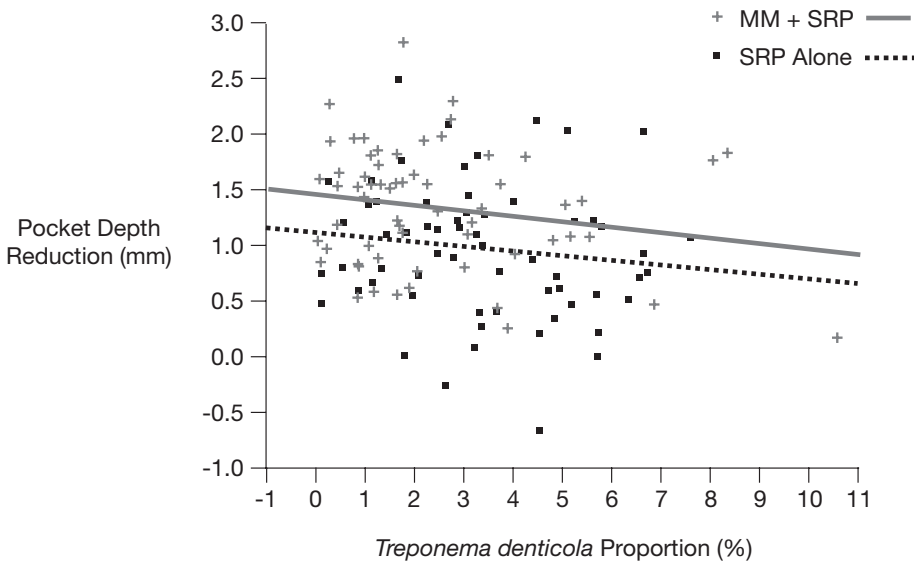


***Figure 3. Pocket depth reduction and Treponema denticola proportions at 30 days for each treatment. MM, minocycline HCl microspheres; SRP, scaling and root planing. BOP, bleeding on probing; CAL, clinical attachment level; PD, pocket depth.***

### *NNT (number needed to treat)*

Comparing MM + SRP to SRP alone, the NNT based on PD change was 6.5 and based on attachment level change was 8.3.

### *Safety*

Seventy-three non-serious adverse events were reported during the study. Subjects in the SRP alone group reported nearly twice as many adverse events as subjects in the MM + SRP group (48 vs 25 adverse events, respectively). Both treatment groups, however, experienced most adverse events at similar frequency. One major exception was oral pain, which occurred three times more frequently in the SRP alone group. The two most frequently reported adverse events in both treatment groups were headache (15% vs 16% in the SRP alone and MM + SRP groups, respectively) and oral pain (30% vs 9% in the SRP alone and MM + SRP groups, respectively). No serious adverse events were reported. Results from the intraoral examination were either considered normal or unrelated to treatment, with the exception of periodontal abscess formation and tooth extraction, which are not unexpected findings in this patient population.

## Discussion

The data from this study indicated that the addition of MM to SRP for the treatment of moderate to severe chronic periodontitis resulted in a significant improvement of PD reduction, a decrease in number of pockets, reduced BOP and improved CAL. The clinical effects of SRP have been well reported in the literature (Isidor and Karring, 1986; Lindhe *et al.*, 1984; Pihlstrom *et al.*, 1983; Ramfjord *et al.*, 1987). Reductions of probing depth and improvement in CAL have been repeatedly demonstrated as well (Badersten *et al.*, 1981; Pihlstrom *et al.*, 1981; Ramfjord *et al.*, 1987). In recent years, the use of locally delivered adjunctive antimicrobial agents to enhance the results of SRP has been extensively evaluated (Carvalho *et al.*, 2005; Cortelli *et al.*, 2006; Faveri *et al.*, 2006; Feres *et al.*, 2001; Hung and Douglass, 2002; Lessem and Hanlon, 2004; Meinberg *et al.*, 2002; Niederman *et al.*, 2002; Page, 2004; Paquette *et al.*, 2004; Renvert *et al.*, 2006; Van Dyke *et al.*, 2002; Williams *et al.*, 2001). A recent comprehensive systematic review article identified adjunctive antimicrobial agents that have been the most extensively studied and met their criteria for inclusion in the review (Bonito *et al.*, 2005). These included chlorhexidine, doxycycline, metronidazole, minocycline, tetracycline, and a group of other antimicrobials. The major probing depth reductions were in the range of 0.25 to 0.50 mm and the major CAL gains were in the range of 0.10 to 0.50 mm. Our findings for PD reduction and CAL gain are very consistent with these results. On average, the addition of MM as an adjunct to SRP resulted in 0.37 mm PD reduction and 0.36 mm CAL gain. Similarly, compared with the probing depth reduction found by Williams and colleagues (2001), who completed a phase III study on MM as an adjunct, our average PD reductions were only 0.02 mm (0.39 mm versus 0.37 mm) less than what they found. Although this was remarkably similar, we reported a statistically significant gain in CAL that they did not find. Their study was also conducted over a 9-month period, but the data reported at three months are still very similar in regard to probing depth reduction.

Even though the clinical data on SRP and local antimicrobial adjunctive agents are extensive, the effects of therapy on the subgingival microflora, and the biofilm they exist within, has not been as well documented. Although some studies have included microbiologic measurements along with clinical measurements (Carvalho *et al.*, 2005; Faveri *et al.*, 2006; Feres *et al.*, 2001), they typically are reported as differences from baseline data. This study was designed to allow comparison of clinical to microbiologic changes. Advances in molecular microbiology allowed for a study with sufficient power to be designed to compare treatment results with microbiologic changes. Haffajee and colleagues (1997) described the primary effect of SRP on the subgingival microflora as a reduction of what is now known as the RCB. Our results confirmed those observations as well and showed an increased beneficial effect to SRP with the addition of MM. The regression analysis used in this study suggested a means to interpret the relationship between clinical and microbiologic responses. For the regression equation between PD reduction and RCB proportions (PD reduction [mm] = -0.027 x RCB [%] + 0.71), complete elimination of RCB (i.e., RCB = 0%), would create an estimated 0.71 mm reduction in PD. This could be considered the maximum PD reduction possible by elimination of RCB. Solving the equation for zero PD reduction, we find that for all values of RCB above 26.6%, PD reduction becomes negative. This value of 26.6% RCB suggests a threshold beyond which tissue repair cannot overcome bacterial pathogenicity. The regression equation between CAL and changes in RCB (CAL changes [mm] = -0.035 x RCB [%] + 1.28) predicts that if RCB were eliminated (i.e., RCB = 0%), a CAL gain of 1.28 mm would be obtained. Considering that a reduction of 1.16 mm by MM + SRP was measured in this study, it seems likely that 1.16/1.28 = 91% of the maximum level of periodontal healing possible was obtained by treatment with MM + SRP, whereas only 0.80/1.28 = 63% of the maximum level of periodontal healing possible was obtained by SRP alone. In addition, this equation predicts that when RCB proportions exceed 36.6%, CAL loss and disease progression will occur. If the effect of the RCB reduction at 30 days is analyzed with clinical end points, it is most strongly correlated with PD reduction (*Table 3*). Additionally, if the correlation of clinical end points and the proportion of each of the RCB are further analyzed, then *T. forsythia* is most highly correlated with this PD reduction (*Table 4*). These data appear to support what is known - a reduction in the subgingival levels of periodontal pathogens leads to a significant improvement in clinical parameters (Haffajee *et al.*, 2006).

A measure currently used in evidence-based dentistry to allow comparison between statistical significance and clinical relevance is the NNT. Originally used in the medical literature (Walter and Irwig, 2001), a modification of the formula can allow an estimation of a positive outcome between a treatment (intervention) group and a control group (Greenstein and Nunn, 2004). That number for a clinically significant PD reduction (≥ 2 mm) at sites with deep pockets (PD ≥ 5 mm) at baseline was 6.5. The literal interpretation of this value is that the clinician must treat 6.5 sites with MM + SRP to achieve one more clinically significant PD reduction than treating with SRP alone. Because the average number of sites with deep pockets was 26 in each subject, 26/6.5 = 4 more sites achieved clinically significant PD reduction in subjects treated adjunctively with MM. The NNT computed for CAL gain was 8.3. The relevance of these numbers must be determined by the treating clinician as related to the benefit for the patient.

Five subgingival samples of bacteria from each subject were obtained at interproximal sites that initially exhibited PDs of ≥ 5 mm at baseline and 30 days. DNA probe analysis, utilizing DNA:DNA hybridization, allowed for identification of 40 bacterial species. Three of these species, *P. gingivalis*, *T. forsythia,* and *T. denticola*, have been highly correlated with the presence of chronic periodontitis (Holt and Ebersole, 2005). These bacteria make up the RCB (Socransky and Haffajee, 2005; Socransky *et al.*, 1998) and are thought to be the primary pathogens in the development of periodontal disease. The levels of RCB were reduced in both test and control groups; however, there was a significantly greater reduction with the addition of MM. This was demonstrated by a reduction in numbers and proportions of RCB. The biofilm initially consisted of 13.45% RCB, but was reduced to 7% RCB, whereas the control reduced the percentage of the RCB in the biofilm from 15% to 10%. This represented a statistically significant difference of 6.49% - 5.03% = 1.46% ($p$ = 0.0005) reduction in RCB for the MM + SRP group versus the SRP alone group. Previous findings have suggested that the microbiological goal of periodontal therapy should be to decrease the quantity of periodontal pathogens to that of healthy patients (approximately 7%) (Teles *et al.*, 2006).

The safety of subgingival MM was well documented in an earlier large clinical trial (Williams *et al.*, 2001). In this study, safety was confirmed by the low incidence of adverse events recorded. A total of 73 non-serious adverse events were reported, most commonly associated with headache and pain of the teeth and gums. Both groups exhibited adverse events at equal frequencies, with the exception of oral pain, which was reported by three times as many subjects in the SRP alone group as in the MM + SRP group. These data suggest that locally applied MM may have reduced the incidence of pain, possibly through the anti-inflammatory effects that have been reported for the tetracyclines as a class (Choi *et al.*, 2004;

Seymour and Heasman, 1995; Zanjani *et al.*, 2006).

In conclusion, the adjunctive effect of MM to SRP led to a significant improvement in all clinical parameters measured (i.e., PD, number of pockets ≥ 5 mm, BOP, and CAL), as well as a decrease in the proportions and numbers of the RCB (*P. gingivalis*, *T. forsythia*, and *T. denticola*). The reduction of these bacteria was significantly correlated with a decrease in PD, particularly the reduction of *T. forsythia*. In addition, the safety of MM was confirmed.

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