Clinical Efficacy of Local Delivery Minocycline Gel for the Treatment of Moderate to Severe Periodontitis

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ABSTRACT

Local Delivery Minocycline Gel for the

Treatment of Moderate to Severe Periodontitis

The Advanced Education Program in Periodontology, the University of Minnesota

Background: Local delivery of antimicrobial agents has been used for many years as an adjunct to treat periodontal disease. The gel form of minocycline local delivery application has an advantage in that it allows multiple sites to be treated with the same syringe loaded with minocycline gel when compared to other modalities on the market. However, the use of this gel form of minocycline has not been clinically proven effective or approved for clinical use in the United States.

Objectives: The objective of this study was to determine the clinical efficacy of a 2.1% minocycline gel as an adjunct to scaling and root planning (SRP) for treating patients with moderate to severe periodontitis. The hypothesis for this study is that patients with moderate to severe periodontitis would have a statistically significant greater increase in clinical attachment (CAL) gain, reduced pocket depth (PD), and reduced bleeding on probing (BOP) when SRP with adjunctive minocycline gel were used when compared to patients treated with sham (SRP alone) and vehicle control (SRP+ vehicle without active ingredient).

Material and Methods: Eligible patients (n=59) had at least ten remaining teeth and each subject had at least four teeth with pocket depths ≥5 - ≤9 mm with BOP at baseline. In addition to baseline, patients were evaluated at three, six and nine months. Enrolled

subjects were randomly assigned to one of three groups: 1) root planing therapy only-sham; 2) root planing therapy and the vehicle control; 3) root planing and minocycline gel. The minocycline gel as well as the vehicle control were administered at 2 weeks and at the one, three and six-month visits. No mechanical debridement as supportive periodontal therapy was performed during this study.

Results: It was found that in the overall study population the minocycline study group, when compared to the sham group, resulted in a greater significant decrease BOP ($p=0.035$) at 3-month, but not when compared to the vehicle control group ($p=0.64$). In sites presenting with the severe form of periodontitis (≥8 mm), there was a statistically significant difference between the minocycline gel group and the sham group with respect to % BOP reduction at 9-months ($p=0.014$). On the other hand, results failed to show any other statistically significant difference between the minocycline gel group and the sham group with respect to other clinical variables including PD and CAL ($p>0.05$). However, minocycline gel did present with statistically significant differences compared to the vehicle control group with respect to the clinical variables.

Conclusion: The minocycline gel as an adjunct to non-surgical periodontal therapy provided a significant favorable clinical effect in decreasing BOP for all moderate to severe periodontitis sites following SRP at the 3-month evaluation. In the severe forms of periodontitis, minocycline gel had an adjunctive favorable effect at 9-months after SRP with respect to %BOP reduction.

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REVIEW OF THE LITERATURE

Periodontitis is characterized by the progressive loss of support and clinical attachment of teeth. It is known that periodontitis is accompanied by inflammation and degradation of the tissues surrounding and supporting the teeth, comprising the gingiva, alveolar bone and the periodontal ligament. In periodontitis, the destruction of the supporting tissues ultimately may lead to tooth loss. Clinically, periodontitis presents with episodes asynchronous, multiple burst with respect to the duration of the disease and pattern of periodontal tissue destruction. The primary etiology of periodontitis is bacterial plaque that accumulates on the tooth surface and under the gingival margin. This bacterial plaque colonizes and forms an organized mass that can shift from a healthy state to pathogenicity. Bacterial composition and the pathogenic potential of plaque can vary from individual to individual, tooth to tooth, and site to site, even in the same individual. Periodontal pockets harbor plaque over time, and if left untreated they will, in a susceptible individual, get deeper and become inaccessible to daily oral hygiene procedures.

It is estimated that at least one third of dentate U.S. adults over the age of 30 have periodontitis[1]. Among periodontitis patients, roughly 22% will clinically present a mild form of the disease and 12.6% will clinically present a moderate or severe form. As the prevalence of periodontitis increases with age, so does the prevalence and extent of attachment loss leading to tooth loss[2].

Introduction

Current evidence supports the fact that periodontitis is a multifactorial chronic disease model. These multiple factors influence clinically observable phenotypes at the individual patient level. It is established in the literature that periodontal pocket depth classification based on severity alone fails to capture important dimensions of an individual's susceptibility to the disease, especially with regard to the complexity of managing moderate to severe forms of periodontitis.

A review by Wolff and colleagues. presented the 'risk assessment model' involving the bacterial factor, as well as the environmental and host factors, leading to and affecting the phenotypic clinical presentation of periodontitis[3]. In this model, Wolff and his colleagues describe how a potentially pathogenic bacteria may also exist in a carrier state which may be compatible with periodontal health. However, if an environmental and/or host risk marker that may negatively affect the host's susceptibility enters into the model, then a specific strain of bacteria may shift the bacterial colony to pathogenicity and lead to periodontal disease, resulting in loss of attachment. The presence or absence of BOP is considered a negative predictor indicator for periodontal disease progression. Although the sensitivity of BOP is around 30%, the specificity of BOP as a negative predictor for periodontal disease presence was found to be 98% as described in a study by Lang and colleagues[4]. These findings are in agreement with previous studies and papers describing the importance of BOP presence or absence as an important marker and as a part of the risk assessment for periodontal disease[5-8].

Treatment of Periodontal Disease

The primary objective of periodontal therapy is to eliminate the inflammatory process resulting in periodontal tissue destruction via the host immune response by altering the composition of the bacterial ecosystem present within the subgingival plaque. Periodontal therapy is composed of both non-surgical and surgical modalities. Mechanical debridement is a widely used non-surgical method to control disease progression, but it can also be used to alter the periodontal environment to a less pathogenic state. This is done by altering bacterial numbers and by modifying the root surface to favor periodontal healing. As of today, the 'gold standard' of non-surgical periodontal therapy involves mechanical scaling and root planing (SRP). SRP is known to be clinically effective[9-11].

A systematic review assessing the clinical outcomes of non-surgical periodontal therapy (SRP) reported overall probing pocket depth (PD) reductions of an average of 1.45 mm and clinical attachment level (CAL) gain of 0.89 mm following root planing[4]. Similar results were consistently reported across other systematic reviews[9,12]. These findings can serve as a reference point when assessing the effectiveness of adjunctive therapies to non-surgical periodontal therapy and their benefit to the overall clinical variable outcomes.

Retrospective studies reported that untreated periodontal disease causes 0.36 teeth to be lost per year, as stated in a study by Becker and co-workers, which followed up on

periodontitis patients[13]. In his study, Becker looked at patients who chose to be left untreated for their periodontal condition, patients that were treated and maintained with supportive periodontal therapy, and patients who chose to be treated and not maintained. Over the 10 year follow up, Becker found that periodontitis was progressive in these patients and that periodontitis can clinically present a rapid progression with a periodontal breakdown if left untreated. With respect to the type of tooth loss, molars were lost most frequently. Overall, there was a difference in annual average tooth loss for the different groups of patients. Becker and his colleagues described that patients who were in need for periodontal therapy but did not follow through therapy lost 0.36 teeth annually compared to periodontally treated patients which were not maintained with supportive periodontal therapy having an annual tooth loss of 0.22. On the other hand, patients who were periodontally treated and well maintained lost only 0.11 teeth annually.

The gingival crevicular fluid (GCF) represents an interstitial fluid which appears in the gingival crevice as a result of a change in the osmotic gradient. This initial, pre-inflammatory fluid is considered to be a transudate which, upon stimulation, becomes an inflammatory exudate[14]. Any substances that are placed into the periodontal pocket are washed out by the GCF, making it difficult for any drug to achieve therapeutic levels and to be maintained within the periodontal pocket[15-17]. In deep pockets with periodontal disease, the GCF flow is increased significantly (44 µl/h) compared to shallow healthy

pockets (only 1-3 µl/h). This phenomenon may reduce the therapeutic effect of any therapeutic agent placed into the periodontal pocket[18].

Antimicrobial Agents Used in Periodontal Therapy

Antimicrobials have been used as adjuncts to periodontal therapy for decades to achieve an increased level of clinical effectiveness. In order to be considered beneficial and effective, antimicrobials should reach the minimal inhibition concentration (MIC) at the site itself and should remain for a long enough time to achieve clinical effectiveness. Systemic antibiotics used as adjuncts have an advantage in that they have the capability to reach across multiple sites at the same time and to decrease either total bacterial count or the amount of a specific strain of bacteria, depending on their mode of action. This use of systemic antibiotics offers both a more affordable adjunct therapy and a less invasive procedure than local delivery agents, but it is more patient dependent. Although there are certainly benefits to the use of systemic antibiotics, they cannot replace root planing for surface modification and for physically removing plaque[19-22] . The disadvantages in using systemic antibiotics are the development of antibiotic-resistant strains of bacteria due to the higher dependency on patient compliance and the potential for adverse reactions with other medications the individual may be on.

The idea of using antimicrobial agents in a local delivery method as a way to boost therapeutic antibiotic doses and concentration in the periodontal pocket was introduced by Goodson and co-workers in the late 70's. An effective locally delivered antimicrobial agent is one that is effective against the target microbial flora and one which exhibits fewer or no undesirable systemic effects. Local delivery agents do not depend on patient compliance. Local delivery agents in the periodontal pocket can attain

a 100-fold higher concentration of the antimicrobial agent in subgingival sites than when administered systemically, with less potential risk of developing resistant strains and super infections. Furthermore, there is a lower risk of adverse systemic reactions.

Kinetics of Drug Release

Along with its advantages, applying an antimicrobial agent in a local delivery system also has its limitations. The GCF washing effect, as well as the high rate of GCF turnover, poses a challenge to maintaining the agent inside the periodontal pocket[23]. The *percentage* of drug that is eliminated per time unit, independent of the concentration, is called first-order kinetics. In contrast, zero-order kinetics is the constant amount (milligrams, etc.) of drug quantity eliminated per unit of time. When drugs are delivered in a controlled release method, the level and time availability of a drug is dependent mainly on the removal rate and the rate of release from the carrier device. The ultimate challenge with local delivery agents is how to maintain the effective therapeutic range; the rate of release of the drug itself, and the rate of the carrier's absorption or breakdown, can affect the efficacy of the drug. A late carrier breakdown will result in a lower amount of total drug availability and the concentration necessary to maintain therapeutic levels.

For drugs administered systemically, the drug concentration in blood plasma increases over time until it reaches a concentration peak. The concentration peak is followed by a decrease according to the drug's half-life after reaching a concentration plateau within the therapeutic range. Low dosages of systemically delivered drugs may

result in less than the necessary therapeutic levels. One of the greatest advantages of using a controlled release agent is that it provides an immediate effective dosage, capable of rapidly reaching the therapeutic level in a specific site which is maintained within the therapeutic range over time utilizing either zero-order kinetic release or sustained release.

Chlorhexidine Local Delivery Antimicrobial Agent Used in Periodontal Therapy

Chlorhexidine (CHX) is an antiseptic agent that was discovered by scientists who were looking for anti-malaria agents, and it has been used for oral application since 1959. In modern times, chlorhexidine's major application objective is the control of dental plaque[48]. Chlorhexidine gluconate is a cationic bis-biguanide, broad spectrum antiseptic, with a positive charge that binds to the negative charge on the bacterial cell wall and salivary glycoproteins. By this binding mechanism, CHX interferes with pellicle formation and bacterial attachment to the tooth surface, as well as altering the osmotic equilibrium which leads to the disruption of bacterial cell integrity. The CHX duration of action is prolonged by its ability to be absorbed by oral structures, and then slowly being released for up to 24 hours[24].

Chlorhexidine was studied extensively with respect to a local delivery subgingival application and its effects on periodontitis[25,26]. Khoo and Newman obtained direct evidence of an effect against subgingival plaque in terms of reducing proportions of spirochetes and motile organisms[27,28]. A study from the Hebrew University in Jerusalem published by Friedman and co-workers looked at chlorhexidine dilacetate in a

mode of sustained release to deal with the challenge of the 'washing effect' by the GCF[29]. Friedman found that embedded chlorhexidine in polymeric films presented a sustained release of the drug over several months.

Soskolne and colleagues studied the efficacy of a chlorhexidine chip (PerioChip), which is composed of CHX in a biodegradable case in a cross-linked hydrolyzed gelatin matrix that is released when in serum, urine and GCF media[30]. Soskolne found that the initial peak concentration of chlorhexidine in the GCF was reached as soon as two hours post chip insertion, results which were maintained over the next 96 hours. Soskolne also reported that the CHX concentration progressively decreased until the study conclusion. The results of the study also showed that CHX was not detectable in any of the plasma or urine samples at any time point during the study, proving no detectable systemic absorption of locally delivered chlorhexidine.

A split-mouth, single-blind study led by Heasman and colleagues looked at the efficacy of local delivery of the 'PerioChip' chlorhexidine sustained release agent as an adjunct to SRP in periodontitis patients[31]. Heasman found that only at the six-month follow-up was there a difference between the test group using PerioChip chlorhexidine adjunct to SRP compared to SRP alone at sites with PD greater than 5 mm at baseline.

A different clinical trial led by Stabholz and colleagues looked at the efficacy of chlorhexidine from an ethyl cellulose-based carrier in a 2-year follow up with a split mouth clinical trial comparing the chlorhexidine sustained release adjunct to SRP and routine maintenance therapy following SRP[32]. In this study the results showed that there

was a significantly greater pocket depth reduction and attachment level gain compared to SRP alone. These results were stable and maintained over a 2-year follow up period.

With an idea similar to the concept of using PerioChip as an adjunct to SRP compared to SRP alone, Jeffcoat and co-workers looked at the clinical variables after using a local delivery chlorhexidine chip compared with SRP alone[33]. In this multi-center study, the results demonstrated doubling in the proportion of sites showing a greater pocket depth reduction of 2 mm or more from baseline at 9 months in the PerioChip + SRP group compared to SRP alone group in sites greater than 5 mm at baseline. Azmak and colleagues looked at the effect that the subgingival controlled release of a chlorhexidine chip had on the matrix metalloproteinase-8 (MMP-8) levels in the gingival crevicular fluid[34]. Azmak reported results showing that there were significant decreases in the GCF MMP-8 levels for the SRP plus PerioChip group compared to SRP alone at sites with PD of 6 to 8 mm at baseline.

On the other hand, Grisi and colleagues reported contradicting findings that the PerioChip did not provide any clinical or microbiological adjunct benefit to that achieved with conventional scaling and root planing alone in a 9-month clinical trial[35]. In another systematic review paper, PerioChip was compared with other local delivery antimicrobials. Addy and colleagues also found contradicting results to previous papers and studies that described a statistically significant positive effect of PerioChip on PD and CAL[36]. Addy reported that the CHX chip did not appear as clinically effective as more specific antimicrobial drugs such as metronidazole or tetracycline when used as

adjuncts to mechanical non-surgical periodontal therapy. Overall, with these contradicting reports, PerioChip studies presented inconclusive and contradictory data to show definitive favorable and reproducible results in treating periodontitis patients either as a sole therapy, or as an adjunct to traditional non-surgical periodontal therapy.

Metronidazole Local Delivery Antimicrobial Agents Used in Periodontal Therapy

The antimicrobial bactericidal agent metronidazole was found to have a favorable effect on the clinical variables of patients presenting with chronic periodontitis[37]. The MIC of metronidazole gel was maintained in the GCF for 24-36 hours. With that concept in mind, the gel needed to be redelivered into each pocket at one-week intervals to generate positive clinical results.

In a paper from the year 2000, a group led by Stelzel looked at the efficacy of 25% metronidazole dental gel as an adjunctive therapy to SRP[38]. Fifty-nine patients with adult periodontitis were followed for a 9-month period in a split mouth design randomized clinical trial. The results showed minor improvement in favor of the application of metronidazole gel as an adjunct to SRP compared to the SRP alone control group. A separate investigation by Ainamo and colleagues performed a multi-center, randomized clinical trial with a split mouth design that compared the monotherapy of metronidazole gel to SRP for PD $\geq$ 5 mm[39]. Ainamo reported no significant differences between the two study groups after 24 weeks.

Tetracycline Local Delivery Antimicrobial Agents Used in Periodontal Therapy

Tetracycline can be produced naturally from a species of Streptomyces or it can be derived in a semi-synthetic fashion[40]. Tetracycline is bacteriostatic antimicrobial agent effective against rapidly multiplying bacteria and gram-positive bacteria. Tetracycline possesses unique non-antibacterial characteristics such as collagenase inhibition, inhibition of neutrophil chemotaxis, anti-inflammatory effects, inhibition of microbial attachment, and root surface conditioning [41]. The antibacterial mechanism of tetracycline is the inhibition of protein synthesis in prokaryotes by binding to 30S ribosomes in both gram-negative and gram-positive microorganisms. Tetracycline acts by preventing attachment of aminoacyl tRNA to the RNA-ribosome complex and it simultaneously inhibits other steps of bacterial protein synthesis, providing a wide spectrum of bacteriostatic activity.

In one of his earlier studies from the 70's, Goodson and co-workers used tetracycline filled hollow fibers placed in the gingival crevice to treat periodontitis[42,43]. Results of Goodson's studies showed that the tetracycline fibers had "a dramatic effect on both the periodontal subgingival microflora and on the clinical manifestations of periodontal disease". The down side of these fibers was that they allowed only a minimal control of drug release as the majority (95%) of the drug was released in the first 2 hours. Drug leakage also negatively affected the therapeutic range of these tetracycline fibers.

Michalowicz and colleagues reviewed periodontal disease recurrence at 3-12 months following various treatment modalities compared to controlled release

tetracycline fibers as adjuncts to SRP [44]. Michalowicz and colleagues found that the sites treated with tetracycline fibers had significantly less disease recurrence (4%) than tetracycline fiber therapy as a sole therapy with no SRP. Maze and co-workers studied a tetracycline-controlled release material following a single application of 35% tetracycline hydrochloride in a glycolic acid gel in healthy adult volunteers[45]. The biodegradable gel was found to be safe and the effective range of bacteriostatic activity against putative periodontopathogens was in the 2-10 *μg/ml* range. This range had a significant antimicrobial efficacy lasting up to 8 days. Friedsen and colleagues studied the efficacy of locally delivered tetracycline strips administered in conjunction with root planing compared to root planing alone[46]. The author reported a significant positive PD and BOP reduction compared to baseline in the test group. The anti-collagenase activity of tetracycline was reported in several animal studies[47-53]. Local delivery tetracycline also inhibited polymorphonuclear leukocyte collagenase activity in vitro; this suggests that locally delivered tetracycline fibers placed into individual periodontal pockets could maintain tetracycline concentration in the GCF, which would then potentially inhibit periodontal tissue breakdown by tetracycline's anti-collagenase activity. Tetracyclines may also have an effect on members of the microbial community that have a lower MIC than other putative pathogens.

Doxycycline Local Delivery Antimicrobial Agents Used in Periodontal Therapy

Doxycycline was patented in 1957 and has been commercially used since 1967. Doxycycline, as is the case for other tetracycline members, is bacteriostatic and it works by preventing bacteria from reproducing through the inhibition of protein synthesis. One of its differences from tetracycline is that doxycycline enters the duodenum for absorption more than tetracycline compounds due to its unstable state at a lower pH.

The local delivery doxycycline commercially available today is Atridox. Atridox is a doxycycline polymer which is an injectable polymer matrix containing 8.5% doxycycline hyclate. Slots and colleagues described how periodontal pathogens have increased antimicrobial susceptibility to doxycycline when compared to tetracycline and other antibiotics [54]. In a 6-month, randomized, multi-center, placebo-controlled study of the efficacy of a combination of systemically delivered doxycycline hyclate 20 mg bid plus locally delivered doxycycline hyclate gel (10%) in PDs ≥5 mm as an adjunct to SRP was compared to SRP plus placebo[55]. Clinical variable outcomes showed that for PD's ≥4 - ≤6 mm, the combination therapy provided significant improvement over the control group for both PD and CAL.

Results of another clinical trial looking at the efficacy of locally delivered doxycycline in a gel form reported promising findings[56]. In this study, there were significant reductions in the number of anaerobic bacteria lasting at least 21 days, which then returned to baseline by 91 days. These findings provided further evidence for a short-term antimicrobial effect with local delivery. Overall, doxycycline gel significantly

reduced the anaerobic population in plaque, but it did not result in a change in either the number of resistant bacteria present or the acquisition of antibiotic resistance.

Eickolz and co-workers used a 15% doxycycline gel as an adjunct to SRP and assessed the relative attachment level gain and pocket depth reduction compared to SRP alone in severe periodontitis patients (PD ≥7 mm)[57]. It was concluded in this paper that adjunctive topical subgingival application had favorable effects with respect to attachment level gain and PD reduction compared to SRP alone. In a multi-center study, the efficacy and safety of doxycycline hyclate (8.5%) delivered through a biodegradable polymer as the sole therapy in patients with moderate to severe periodontitis was compared to SRP alone[58]. The results of this study demonstrated a mean 9-month CAL gain of 0.8 mm for the doxycycline group and 0.7 mm for the SRP group in one study center, and 0.8 mm and 0.9 mm respectively in the other center. With respect to PD reduction, the results of this study showed a mean PD reduction of 1.1 mm for the doxycycline group and 0.9 mm for the SRP group in one center, and 1.3 mm for both the test and control group in the second center. The results of this study suggested that doxycycline as a sole therapy has a similar clinical efficacy and a clinical equivalence to SRP.

A small split mouth study by Martorelli and colleagues of 11 individuals with type I diabetes with baseline PD greater or equal to 5 mm compared the outcomes of SRP only to doxycycline gel plus SRP and SRP plus placebo gel[59]. Martorelli found there was greater PD reduction and CAL gain in the doxycycline gel group than in the control

group. The authors concluded there was an overall clinical benefit of using the adjunctive doxycycline in diabetes mellitus type I patients with chronic periodontitis.

Minocycline Local Delivery Antimicrobial Agents Used in Periodontal Therapy

Minocycline is a semi-synthetic antimicrobial agent from the tetracycline family. A number of studies in the literature advocate minocycline as a more effective antibacterial agent than tetracycline in its inhibition of gram-negative facultative anaerobes, and that minocycline has a combined effect of increased absorption and a higher concentration in the GCF when administered systemically. These characteristics make minocycline a more preferable antibiotic in a local delivery system with potential for better clinical efficacy in the treatment of periodontal infections.

Clinically, with respect to periodontitis, a review by Van der Kerckhover focused on minocycline ointment application as an adjunct to SRP[60]. It was concluded in the review that minocycline ointment had a positive significant result in reducing bacterial populations and in eliminating motile organisms. The efficacy of 2% minocycline ointment was evaluated by Van Steenberghe and co-workers in a randomized clinical trial in individuals exhibiting moderate to severe periodontitis[61]. In this study, the authors evaluated the antimicrobial activity of minocycline against *A. actinomyctemecomitans, P. intermedia,* and *P. gingivalis* which are known to be major periodontal pathogens. Van Steenberghe and co-workers found that in pocket depths greater than or equal to 5 mm with CAL loss of greater than or equal to 3 mm at baseline, there was a clinically

favorable response to minocycline ointment. Probing depths were reduced at 42% of the test sites (SRP+ 2% minocycline ointment), compared to 28% reduction of the control sites (SRP only).

A different local delivery system of minocycline was introduced by Jones and colleagues[62]. In this study, Jones and colleagues looked at the efficacy of a minocycline periodontal powder microencapsulated in a biodegradable polymer, poly glycolide-co-dl-lactide, in periodontitis sites as an adjunct to SRP. The results of this study showed that the prevalence of *P. gingivalis* and *P. intermedia* decreased significantly when the microencapsulated minocycline microspheres were used as an adjunct to SRP in sites with greater than or equal to 5 mm pocket depths.

A similar study by Williams and co-workers looked at the minocycline microspheres delivery system as a sustained delivery mechanism to treat sites with 6 to 9 mm pocket depths exhibiting BOP[63]. In this study, three parallel treatments: SRP alone, SRP and a vehicle control, and SRP with the minocycline microspheres; were put to the test in moderate to advanced periodontitis sites. The group treated with minocycline microspheres showed substantially greater probing depth reduction than either SRP alone or the SRP and the vehicle control. The results showed that clinical efficacy reached the level of significance in as early as one month into the study. This level was reported to be maintained throughout the trial and was independent of patient smoking status, age, gender, or baseline disease level[64]. Van Dyke and colleagues in a series of studies, also found substantially greater reductions in PD and CAL gain at each post-operative time

point in the group where minocycline microspheres were used as an adjunct to SRP, compared to SRP alone[65,66].

McColland and co-workers compared local delivery of 2% minocycline gel to conventional subgingival debridement as part of a supportive periodontal therapy maintenance program[67]. The results presented in this pilot study failed to show a significant difference between minocycline gel as a sole therapy and periodontal non-surgical therapy alone. Interestingly, in this study, the prevalence of *P. gingivalis, T. forsythia, T. denticola, A. actinomycetemcomitans, P. intermedia*, and *P. nigrescens* remained similar for both study groups. This further emphasizes the importance of mechanical debridement as an essential part of periodontal therapy.

A recent pilot study, comparing the effects of antimicrobial photodynamic therapy and local administration of minocycline on clinical, microbiological and inflammatory markers of periodontal pockets, was conducted in Japan[68]. The results of this study showed that minocycline administered locally had improved clinical, microbiological, and crevicular cytokine levels in the periodontal pocket. However, photodynamic therapy alone did not show any beneficial clinical effects. In this study, local delivery minocycline had shown a significant decrease in scores for the clinical outcomes of bacterial counts, as well as pro-inflammatory cytokines, such as interleukin 1β.

With similar objectives in mind, but with contradictory results, a study published by Tabenski and colleagues looked at biodegradable minocycline microspheres compared to photodynamic therapy as an adjunct to SRP[69]. This study presented results with

significant improvements in both clinical and microbiological parameters, but the study results did not differ between study groups in a statistically significant manner. It was noted then that efficacy of SRP did not seem to be improved by the local delivery of minocycline microspheres, nor by photodynamic therapy with respect to periodontal clinical variable outcomes.

Inflammatory processes around dental implants can result in the loss of supporting bone to such an extent that it could lead to implant failure. Renvert and colleagues assessed the clinical and microbiologic outcomes of repeated local delivery of 1mg of minocycline microspheres around implants exhibiting peri-implant inflammation, as compared to chlorhexidine gel[70]. Thirty-two subjects with at least one implant with PD $\geq$ 4 mm, combined with bleeding and or exudate upon probing and the presence of putative pathogenic bacteria suggestive of peri-implant inflammation, were included in the study. The use of local delivery minocycline resulted in a significant improvement in probing depths compared to chlorhexidine gel. For the deepest sites of the minocycline treated implants, the mean probing depth reduction was 0.6 mm at 12 months.

Generally, tobacco has an adverse effect on the healing of periodontitis and is a strong modifiable risk factor and predictor of future disease. There is strong scientific evidence that smokers are 2.7 times more likely to have periodontal disease than non-smokers[71]. Tobacco-use has an overall negative effect on blood vessels and is an overall tissue irritant, which leads to a delay in tissue healing. Heavy smoking (>10 cigarettes per day) has been associated with an increased rate of disease progression. Smoking or

tobacco-use status represents a key parameter in the risk assessment for an individual to develop severe periodontitis and therefore makes the smoking population of interest for periodontal research[72-78].

Paquette and colleagues looked at local delivery of minocycline microspheres in tobacco smokers exhibiting clinical signs of moderate to severe periodontitis[79]. At 9-months, smokers treated with SRP and adjunctive minocycline microspheres exhibited a mean pocket depth reduction of 1.19 mm from baseline, compared to 0.90 mm for smokers treated with SRP only. This suggests an additional benefit of local delivery minocycline in smokers with moderate to severe periodontitis. A more recent systematic review addressing whether the use of local or systemic antimicrobials would improve clinical results of non-surgical periodontal therapy for smokers with chronic periodontitis[80]. The meta-analysis resulted in a support of use of adjunctive local antimicrobials in a smoking population exhibiting chronic periodontitis. The local delivery of antimicrobials resulted in a superior clinical response with respect to PD reduction and CAL gain when compared to systemic antimicrobials as adjuncts to SRP.

Statement of the Problem

Non-surgical periodontal therapy provides limited clinical improvement in individuals with moderate to severe periodontitis. The use of adjunctive systemic antibiotics with non-surgical periodontal therapy has been known to be effective for many years. Systemic administration of antibiotics does add to improvement in clinical

variables, but it may cause undesirable adverse effects such as the development of resistant bacterial strains, yeast superinfection, and gastro-intestinal upset. Also, in patients that are on other systemic medications, there is always a potential for a cross adverse reaction. Moreover, systemically administered antibiotics have the additional challenge of keeping a sustained therapeutic concentration in the periodontal pocket. Local delivery agents, however, offer the use of a lesser dosage of the drug with a more potent concentration at the site of treatment along with a minimal risk of a systemic adverse effect.

Rationale for the Study

The purpose of this study is to test the clinical efficacy of a gel form of minocycline for local delivery in treating individuals with moderate to severe periodontitis. A single syringe of the gel allows multiple periodontal pockets to be filled quickly, minimizing treatment time, discomfort to the patient, and expenses to both dentist and patient when compared to the minocycline microspheres. Owing to the gel nature of the minocycline formulation, pockets are dosed based on their size allowing for flexibility with respect to the number of sites treated and the amount of locally delivered agent applied. The flexibility of this minocycline local delivery agent can accommodate for differences in the physical and anatomical characteristics of the periodontal pocket.

Hypothesis

The hypothesis for this study is that individuals who have clinically exhibiting sites with moderate to severe periodontitis will benefit from treatment with local delivery minocycline gel as an adjunct to SRP. The use of minocycline gel as an adjunct therapy at these sites will result in a statistically significant reduced PD, reduced BOP and increase in CAL gain, when compared to patients treated with a SRP alone (sham) and vehicle control (SRP+ vehicle without active ingredient).

MATERIAL AND METHODS

Study Subject Population

Fifty-nine subjects from the University of Minnesota that met the inclusion criteria were included in our study. A selection eligibility criterion was that individuals had to present with moderate to severe chronic periodontitis at baseline (≥5 - ≤9 mm). The subject inclusion and exclusion criteria are shown below.

Inclusion Criteria

1. Patients had to be 18 years of age or older.
2. Patients had to have read, understood, and signed a consent form.
3. Patients had to be able and willing to follow study procedures and instructions.
4. Patients had to have moderate to severe periodontitis with at least 10 remaining teeth, out of which at least four teeth had pocket depths of ≥5 mm and ≤9 mm with bleeding on probing in these pockets.

Exclusion Criteria

1. Patients who had periodontal therapy, surgical or non-surgical, within 6 months prior to enrollment were not eligible.
2. Patients who had clinically significant or unstable medical diseases which might compromise healing, such as diabetes mellitus, hepatic insufficiency of any degree, connective tissue disorders, or who were under treatment where the medication may affect healing (e.g., cancer chemotherapy) were not eligible.

3. Patients with a heart murmur, history of rheumatic fever, valvular disease or artificial heart valves, prosthetic joint replacement, or any other condition requiring antibiotic prophylaxis prior to dental appointments were not eligible.
4. Patients who had been treated with oral antibiotics within 3 months prior to enrollment were not eligible.
5. Patients treated with medications known or suspected to affect periodontal status, including cyclosporine, warfarin, non-steroidal anti-inflammatory drugs, phenytoin, or calcium antagonists, within 1 month of screening were not eligible. Selective use of daily low dose aspirin was permitted in patients who were eligible.
6. Patients who were pregnant, as determined by a positive urine test, or patients who were lactating were not eligible.
7. Female patients who were of childbearing age, except those who were using a hormonal barrier method of birth control, were not eligible.
8. Patients with an allergy to tetracycline or reported serious adverse reactions to minocycline were not eligible.
9. Patients with severe dental disease including caries or any condition likely to require antibiotic treatment during the trial and/or severe untreated dental disease requiring extensive restorative or surgical treatment during the trial were not eligible.

10. Patients with active infections such as HIV, hepatitis, or tuberculosis were not eligible.
11. Patients under treatment for cancer, type IV heart disease, or end stage renal disease were not eligible
12. Patients who had taken an investigational drug within 30 days of enrollment were not eligible.
13. Patients requiring anticoagulation therapy of any type for any reason were not eligible.
14. Patients treated with oral retinoid medications for skin conditions were not eligible.
15. Teeth with pockets surrounding a tooth which had root canal treatment were not eligible.
16. Teeth with pockets in close proximity to a dental appliance were not eligible.
17. Teeth with pockets at the gingival border of an implant were not eligible.
18. Patients with localized aggressive periodontitis were not eligible.

Sham Control, Vehicle Control Minocycline Gel Formulation

Sham control was administered in the same manner as the other treatment groups. An empty syringe was used to mimic the application of the minocycline gel after completion of SRP at baseline.

Vehicle control treatment was the administration of the gel without the active minocycline medication after completion of SRP at baseline.

Minocycline gel (active antimicrobial agent) is a yellow-colored gel containing minocycline hydrochloride, together with hydroxythelcellulose, magnesium chloride, compolymer of actylates and methacrylates, triacetin and glycerol. The minocycline hydrochloride is equivalent to 2.1% minocycline hydrochloride, which is equivalent to 10.5 mg minocycline in a 500 mg gel carrier. It was supplied in a disposable polypropylene applicator.

Study Timeline and Procedures

Study timeline and procedures completed for each subject in this study are shown in Table 1. At the screening visit, full mouth CAL and PD measurements were recorded, and the subjects were then randomly assigned to one of the three study groups: sham, vehicle control and minocycline gel. The dental operators were periodontists who performed scaling and root planing and administered one of the three different treatment modalities to each patient at baseline, 14 days, and at months 1, 3 and 6. There was at least one trained and standardized operator responsible for all aspects of the dental and medical management of patients. Dental examiners for this study were trained and calibrated and were not the same individuals as the dentist providing therapy. Dental examiners were kept blinded to the patient treatment assignments and were not present at

the time of treatment. Dental examiners were responsible for all clinical evaluations including measurements of PD, CAL and the BOP assessment.

Study Groups; Sham Control, Vehicle Control and Minocycline Gel

Group 1 (SRP + sham) had SRP at baseline plus sham treatment with an empty syringe. Group 2 (SRP + vehicle control) and group 3 (SRP + Minocycline gel) had SRP at baseline plus the administration of the vehicle control or the minocycline gel, respectively. After bleeding diminished following SRP, each treatment site was thoroughly rinsed with water, air dried, and isolated with cotton rolls. For group 1 patients (sham), the tip of an empty syringe was applied to the gingival margin as the plunger was depressed into the pocket simulating the application of the gel. For patients of the other two study groups, the tip of the syringe used to dispense the vehicle control or minocycline gel was inserted subgingivally at the treatment sites to the base of the pockets. The pocket was slowly filled drawing the tip of the dispenser coronally.

The syringes, whether containing the vehicle control or the minocycline gel, contained adequate material to fill multiple sites and there was the same quantity of gel (500 mg) in each syringe. The minocycline gel syringe contained 10.5 mg of minocycline in 500 mg gel which could be used to treat multiple pockets in the same patient. The use of a periodontal dressing or any other tissue adhesive was not permitted with any patient.

Demographic Data and Tobacco-Use

Demographic data and tobacco-use for enrolled subjects at the screening appointment are shown in Table 2. Each patient's birth date, gender, ethnicity and smoking status was recorded. Previous periodontal therapy information was also collected and recorded. Medical histories were obtained at each visit from all patients, as well as current medication and the type, dose, and duration of use. Patient vital signs were measured at baseline and at the final visit, including oral temperature, pulse and blood pressure.

Clinical Measurements

At each study visit (screening, baseline, day four, day fourteen, and months three, six and nine), a clinical evaluation of the patients extra and intra-oral structures was performed. A clinical examination of the face, lymph nodes, lips, buccal mucosa, tongue, hard and soft palate, gingiva, floor of the mouth, edentulous ridges and teeth was performed. Abnormal findings were described and recorded, noting location, size, onset, and associated symptoms. The periodontal clinical assessment included pocket depth, CAL, BOP, and recession. All periodontal probing measures were rounded to the lowest mm. The following clinical parameters were measured:

1. Pocket depth (PD), defined as the distance in mm from the gingival margin to the base of the sulcus/pockets on six surfaces/tooth, except for third molars.

2. Recession, defined as the distance in mm from the gingival margin to the CEJ. If the gingival margin was apical to the CEJ, it was given a negative value.
3. Bleeding on probing (BOP), defined as the presence or absence of bleeding on probing as recorded 10 seconds after probing of each quadrant.
4. Clinical attachment level (CAL), defined as the calculated difference between PD and the distance from the gingival margin to the CEJ.

The time frame for each patient to complete their scheduled visits is shown in the illustration on the following page.

Calibration of Clinical Examiners

Calibration of examiners and standardization of all operators occurred at a time prior to initiation of the study. All examiners were required to obtain a high level of reproducibility. Clinical parameters were measured using a University of North Carolina manual periodontal probe. Six sites per tooth were assessed: mesio-buccal, buccal, distobuccal, mesio-lingual, lingual, and distolingual.

Timeframe for Each Patient to Complete Scheduled Visits

Visit	Time window
Baseline	Within 4 days of scheduling, within 30 days of screening
Four day visit	Within 2-6 days of baseline treatment
Two week visit	14 days +/- 2 days from baseline
Four week visit	30 days +/- 5 days from baseline
Three-month visit	90 days +/- 14 days from baseline
Six-month visit	180 days +/- 14 days from baseline
Nine-month visit	270 days +/- 14 days from baseline

Clinical Variable Outcomes

The primary objective of this study was to determine the adjunctive clinical effects of minocycline gel, when compared to the two control groups, with respect to the CAL, BOP, and PD levels. Sites exhibiting a PD increase of 3 mm or more between visits were identified as 'rescue sites'. These rescue sites were treated locally by SRP and other therapy as determined necessary by the study periodontist. Rescue sites were discontinued from the study in terms of the primary endpoint if the breakdown occurred in a treatment site. The last PD value recorded before the breakdown was used in the primary objective calculations.

Statistical Analysis

Descriptive statistics were used to compare the baseline characteristics of patients by treatment group. Continuous measures were summarized with means and standard errors (SEs), and comparisons between treatment groups were made using one-way analysis of variance, which is analogous to the two-sample t-test for more than two groups. Categorical measures were summarized with counts and percentages, and comparisons between treatment groups were made using Fisher's exact test. Similar comparisons by treatment group of the clinical outcomes at baseline (PD, CAL, and percent BOP) were made.

To address the primary research question, PD, CAL, and percent BOP were compared between treatment groups at each follow-up visit (three, six, and nine months post-baseline). Two comparisons were considered: comparison of the clinical measure at follow-up between treatment groups and comparison of the *changes* since baseline in the clinical measure. Additionally, the percentages of sites PDs which improved by 2 or 3 mm were analyzed. The responses within treatment groups were summarized using the mean and SE. Comparisons across groups were performed using a one-way analysis of variance.

In all analyses, the clinical outcomes were analyzed at the patient level. That is, for each patient, outcomes were averaged across sites to obtain the patient-level measurement.

In addition to comparisons by treatment group for all patients, subgroup analyses were performed on the basis of baseline PD. The first subgroup consisted only of sites with

baseline PD of ≥5 mm and ≤7 mm and the second subgroup consisted only of sites with baseline PD of ≥8 mm and ≤9 mm. For these subgroup analyses, the same patient-level averaging was performing using those sites with the appropriate baseline PDs.

p-values less than 0.05 were considered statistically significant. No formal corrections were made for the testing of multiple outcomes.

RESULTS

Demographic Variables

The demographic data and tobacco-use for enrolled subjects collected at the screening appointment is shown in Table 2. There were of 59 subjects (sham-n=19, vehicle control-n=20, minocycline gel-n=20) enrolled in this clinical trial, with 58 subjects completing the study. There were no statistically significant differences ($P > 0.05$) between the three study groups with respect to demographic variables (gender, age, race nor tobacco-use) at the screening appointment.

Clinical variables

Mean clinical variables for all sites evaluated (≥5 mm) in patients for each of the three study groups at baseline are shown in Table 3. There were no statistically significant differences between the groups ($p > 0.05$). For the primary outcome variable of PD; the sham, vehicle control and minocycline gel groups had PDs of 5.82 mm, 5.65 mm, and 5.68 mm, respectively at baseline.

Mean clinical variables for all patients and the change in clinical variables from baseline at 3 months are presented in Table 4. There was a statistically significant difference between the study groups with respect to %BOP reduction ($p=0.042$). With respect to the other clinical variables listed, there were no statistically significant differences ($p>0.05$).

Mean clinical variables for all subjects and the changes in clinical variables from baseline at 6 months are presented in Table 5. There were no statistically significant differences between the groups with respect to all clinical variable outcomes at this time point of the study. The %BOP reduction that was statistically significantly different between the study groups at the 3-month time point was no longer significantly different at the 6-month time point.

Mean clinical variables for all subjects and the changes in clinical variables from baseline at 9 months are presented in in Table 6. There were statistically significant differences with respect to %BOP and %BOP reduction from baseline ($p=0.03$ and $p=0.03$, respectively). Again, the minocycline gel group had the lowest %BOP levels (32.50%) compared to both the sham group (40.84%) as well as compared to the vehicle control group (49.63%). Moreover, the minocycline gel group had the greatest %BOP reduction (62.95%) compared to the sham group (52.35%) and the vehicle control group (46.32%).

Baseline PD ≥5 - ≤7 mm

Mean clinical variables for patients with PDs ≥5 - ≤7 mm at baseline are presented in Table 7. There were no statistically significant differences with respect to PD, CAL and BOP ($p>0.05$). Similarly, clinical variables at 3 months (Table 8) and 6 months (Table 9) did not yield any statistically significant differences for the mean

clinical variables and mean clinical variable change from baseline for the subjects enrolled in each of the three study groups with baseline PDs ≥5 -≤7 mm.

Mean clinical variables and clinical variable changes at nine months for subjects with baseline PDs ≥5 - ≤7 mm is presented in Table 10. For the clinical variable outcomes, there was a statistically significant differences between the study groups with respect to %BOP (p=0.05) and a trend towards statistically significant differences in %BOP reduction from baseline (p=0.06). At the 9-month time point, the minocycline group had the least %BOP (32.35%) compared to the sham group (39.39%) and the vehicle control (48.25%). The %BOP reduction at 9 months was greatest in the minocycline gel group compared to the sham and vehicle control groups (62.85%, 54.40%, 47.83%), respectively.

Baseline PD ≥8 mm

Demographics and tobacco-use in each study group for patients with PDs ≥8 mm at baseline are presented in Table 11. Overall, there were fewer patients in each study group who had sites with baseline PDs of 8-9 mm. Ten subjects in the sham control group, ten subjects in the vehicle control group, and thirteen subjects in the minocycline gel group had at least one site with a PD of 8-9 mm at baseline. No statistically significant differences were found between the different study groups with respect to the demographic variables at baseline (p>0.05).

Mean clinical variables for subjects with PDs ≥8 mm at baseline are presented in Table 12. There was a statistically significant difference between the groups with respect to the clinical attachment levels noted for these sites. CAL baseline level differences for the minocycline gel group (7.02 mm) compared to the sham (8.06 mm) and the vehicle control (6.71 mm) groups were noted to be statistically significant ($p < 0.05$).

Mean clinical variables and clinical variable changes at three months from baseline for subjects with PDs ≥8 mm are presented in Table 13. There were no statistically significant differences with respect to the different clinical variables ($p>0.05$). The mean clinical variables and clinical variable changes at six months from baseline for subjects with PDs ≥8 mm is presented in Table 14. With respect to %BOP reduction from baseline, there was a trend towards a statistically significance difference ($p=0.06$) between the groups at the 6-month visit, where the minocycline gel group (59.07%) showed more %BOP reduction compared to the sham group (41.61%) and to the vehicle control (17.02%). Also, at 6 months there was a statistically significant difference between the different study groups with respect to the percentage of sites that improved by 3 mm ($p=0.02$): the minocycline gel group (71.70%) had the greatest percent of PD reduction by 3mm compared to the sham (40.07%) and to the vehicle control group (30.36%).

Mean clinical variables and clinical variable changes at 9 months from baseline for subjects with PDs of ≥8 mm are presented in Table 15. There was a statistically significant difference between the different study groups with respect multiple clinical

variables, including PD, CAL, and BOP levels. A the 9-month visit, the minocycline group had a mean PD level of 4.87 mm, compared to the sham group (5.99 mm) and the vehicle control (6.19 mm) group, (p=0.03). With respect to PD reduction, the minocycline group had a 3.27 mm PD reduction compared to the sham (2.34 mm) and the vehicle control (1.88 mm) groups, (p=0.03). With respect to CAL gain, there was also a statistically significant difference at 9 months between the study groups: the minocycline group had 2.71 mm of CAL gain, compared to the sham (2.18 mm) and the vehicle control (1.35 mm) groups, (p=0.017). The %BOP, as a clinical variable for this study, demonstrated a statistically significant difference between the study groups. Again, the minocycline group had 36.81% BOP compared to the sham (73.95%) and the vehicle control (68.78%) groups, (p=0.04). Moreover, %BOP reduction at 9 months showed a statistically significant differences between the different study groups. The minocycline gel group had a 63.19% BOP reduction compared to the sham (17.12%) and the vehicle control (21.96 %) groups, (p=0.05). Another clinical variable that yielded statistically significant differences between the study groups was the % of sites to have improve PDs by 3 mm. The minocycline gel group had 62.91% of sites that were reduced at the nine month visit by 3 mm or more, compared to the sham (33.8%) and the vehicle control (21.43%) groups, (p=0.03).

The p-values intragroup analysis for statistically significant findings of the various clinical variables and clinical variable changes are presented in Table 16. With respect to %BOP, at 3 months there was a statistically significant difference between the

minocycline and the sham groups (p=0.03) for both moderate and severe periodontitis sites grouped together, favoring the minocycline gel group. At 9 months the %BOP was statistically significant only between the minocycline gel and the vehicle control groups, again for both moderate and severe periodontitis sites grouped together (p=0.02). Regarding %BOP reduction from baseline, for all sites there was a statistically significant difference between the minocycline gel and the vehicle control groups (p=0.02). With respect to the percentage of sites that had PD levels improved by 2 mm was also evaluated, there was a statistically significant difference between the minocycline gel and the vehicle control groups for sites ≥8 mm at nine months from baseline (p=0.02). Similarly, PD reduction for sites ≥8 mm at nine months from baseline was statistically significantly different for the minocycline gel group as compared to the vehicle control group, favoring the minocycline gel group (p=0.03). With respect to CAL gain, there was a statistically significant difference at 9 months from baseline between the minocycline and the vehicle control groups, favoring the minocycline gel group (p=0.01). The %BOP reduction was statistically significantly different for sites ≥8 mm at 9 months from baseline between the minocycline and the sham groups (p=0.02 and p=0.14, respectively), favoring the minocycline group. Lastly, for the percentage of sites that improved by 3 mm from baseline of sites ≥8 mm, there was a statistically significant difference only between the minocycline group compared to the vehicle control group, again favoring the minocycline group (p=0.03).

DISCUSSION

The aim of this randomized control clinical trial was to determine the efficacy of locally delivered minocycline gel as an adjunct to SRP in moderate to severe forms of chronic periodontitis. In this study, all three study groups were well randomized with no statistically significant differences between the study groups at baseline with respect to demographics, clinical variables, or tobacco-use duration and amount. This study was originally a part of a multi-center research project. This thesis is a description and analysis of the data and findings from a portion of the patients evaluated at the University of Minnesota Clinical Research Center.

Unfortunately, we do not have the data from the other study centers in order to use the power analysis calculated for this study. The fact that we have only part of the data with a limited number of subjects and not the full power intended for this multi-center study should be noted and disclosed in advance. That being said, we can still extract trends and, even in this limited group of subjects, detect statistically significant differences between SRP and SRP + minocycline gel therapy. The results of this study can serve as a pilot study for future investigations of this adjunctive periodontal therapy modality.

Overall, fifty-nine subjects were enrolled in the study. Fifty-eight subjects completed the entire protocol and were followed for 9 months. A total of 658 teeth with 1,464 sites were evaluated at baseline which had PDs ranging from ≥5 mm to ≤9 mm. When study subjects exhibited a clinical manifestation of moderate to severe

periodontitis, there was a statistically significant difference in favor of the minocycline gel group compared to SRP alone (Sham group) with respect to %BOP at the 3-month time point ($p=0.04$). These findings support our hypothesis that sites with moderate to severe periodontitis would benefit from treatment with locally delivered minocycline gel as an adjunct to non-surgical periodontal therapy. The reduction in %BOP at the 3-month visit can serve as support for the use of the minocycline gel as an adjunct to non-surgical periodontal therapy with respect to slowing and positively altering periodontal disease progression. This may indicate and support the use of minocycline gel as part of a supportive periodontal therapy program for moderate to severe periodontitis affected individuals.

For sites exhibiting the severe form of periodontitis with baseline PDs of ≥8 mm, the post-hoc intragroup analysis presented a statistically significant difference between the minocycline gel group and the sham group with respect to %BOP reduction at the nine-month time point only. This interesting finding can be attributed to the repetitive effect of the application of minocycline gel throughout the study, yielding a statistically significant difference compared to SRP treated sites. With respect to other clinical variable outcomes, the minocycline gel group yielded statistically significant differences only when compared to the vehicle control group. These finding again show that the gel by itself without minocycline does not contribute to a clinical improvement. It is the minocycline agent then, in the gel form, in a local delivery system which presents the

adjunctive effect as shown in clinical outcomes (PD, CAL, %BOP) in sites with a clinical manifestation of moderate to severe periodontitis.

The lack of an adjunctive benefit to moderate chronic periodontitis sites is in contradiction to the results of the study by Williams and his co-workers, where the investigators looked at the efficacy of minocycline biodegradable microspheres[33,63]. As they reported, a statistically significant pocket depth reduction of sites ≥ 5 mm at baseline exhibited as much as 0.24 mm additional PD reduction in minocycline microspheres compared to placebo (SE of 0.04). In our study, at sites ranging from ≥5 - ≤7 mm at baseline, there was a 0.28 mm additional PD reduction in minocycline gel treated sites compared to the sham group at 9-months. These results failed to reach the level of statistical significance, perhaps due to lack of power ($p > 0.05$). Williams reported an odds ratio of 1.59 and 2.86 for baseline pocket depths of ≥ 5 mm and ≥ 6 mm, respectively to be reduced to ≤4 mm. In our study, the odds ratio was 1.13 and 1.56 for ≥5 - ≤7 mm and ≥8 mm, respectively (Data not shown). The differences in these results may again be attributed to the much higher power and greater number of sites in the Williams study (748 subjects compared to our 58 subjects). In an attempt to find the 'critical PD level' indicating the clinical use of minocycline gel, statistics were performed for different pocket brackets for the different study groups. For PD levels of ≥5 - ≤6 mm and ≥7 mm (Data not shown), there was no statistically significant difference between the three study groups with respect to the clinical variable outcomes: PD, CAL, and BOP.

Other investigators have also examined the effect of subgingival, local delivery antibiotic formulations on subgingival bacteria[81]. Overall, local delivery antibiotics have shown positive effects not only on clinical variables, but also on the bacterial flora. Upon local delivery antimicrobial application, the subgingival numbers of cocci increased, and the percentage of pathogenic motile rods and spirochetes has been shown to decrease compared to baseline levels.

Concentrations of locally delivered minocycline in the gingival crevicular fluid remain at clinically effective levels for at least 7-10 days. Patients receiving 100 mg of minocycline systemically had a gingival crevicular fluid concentration as low as 4.3 µg/ml[82]. One dose of slow release local delivery minocycline can produce GCF levels of 340 µg/ml - a much higher concentration of minocycline and much more potent than systemic minocycline. Minocycline MIC for bacteria associated with chronic inflammatory periodontal disease ranged from 0.03 to 32 µg/mL. Since systemically administered antibiotics cannot reach such high levels of concentration in the GCF, it is unlikely that these bacterial pathogens can be affected substantially enough to create a more clinically favorable result with respect to periodontal clinical variables in moderate to severe periodontitis affected sites.[83].

We did not have supportive periodontal therapy throughout the study. The clinical responses reported in this thesis are in agreement with other published literature, indicating local delivery antibiotics to be ineffective as a sole periodontal therapy. It would be reasonable to say that if supportive periodontal maintenance was performed in

this study every 3-4 months it would have enhanced the clinical response at sites receiving minocycline gel as an adjunct to SRP, similar to the % BOP reduction we reported at the 3-month time point.

Clinical attachment was a major clinical outcome variable sought in this study as an important factor in determining clinical success. In both moderate and severe chronic periodontitis sites, there was no statistically significant CAL difference between any of the study groups at all time points, including the nine-month visit. According to the results presented in this investigation, minocycline gel as an adjunct to SRP did not present superior improvement in CAL gains compared to SRP alone, and it did not provide additional improvement to the clinical results of treated teeth.

The literature reports a mean average of 0.8 mm additional positive CAL gain when local delivery antimicrobial agents are used as adjuncts to mechanical instrumentation[84]. A note should be made with respect to these finding that there was a statistically significant difference between the study groups at baseline with respect to CAL. The CAL levels of the minocycline gel group had less CAL loss than the sham group, so the sites which had minocycline gel had less potential for CAL gain. Although the intra group post-hoc analyses did not reach the level of a statistically significant difference between the minocycline gel group and the sham group with respect to CAL gain, we did have 0.54 mm of more mean CAL gain in favor of the minocycline gel group.

In this study, the clinical response to minocycline gel was not distinguished between molars and single rooted teeth. It is well established from the literature that molar teeth had an average 1.0 mm deeper probing depth than single rooted teeth in periodontitis patients[85]. Pihlstrom and colleagues, in a longitudinal study, found that molars with initial probing depths of 4-6 mm responded less favorably to periodontal therapy than single rooted teeth with similar baseline pocket depths. The differences in the clinical response to non-surgical periodontal therapy may be attributed to furcation involvement. We cannot exclude the positive nor negative effect molars had on clinical variable outcomes in response to the different therapies in this study.

The assumption that all periodontitis patients are equally susceptible to periodontitis is no longer accurate and it is a misconception that is oversimplified. The paradigm that disease severity is a function of magnitude and of duration of exposure to bacteria is no longer completely accurate. It is becoming more and more evident that the idea that we can prevent and treat chronic periodontitis predictably as long as we have adequate bacterial control is a misconception. It is well established that adults with moderate to severe generalized periodontitis and individuals that are missing teeth are more prevalent in specific subsets of the population.

As of today, there are several slow-release devices that deliver anti-microbial drugs directly into the periodontal pockets. The use of these agents, such as Atridox (high concentration of doxycycline), Arestin (biodegradable minocycline microspheres), and PerioChip (Chlorhexidine), provides a long-lasting, high concentration of the active drug

placed directly into the periodontally involved sites. Although these antimicrobial agents differ with respect to their application technique and the concentration of the drug, there are randomized clinical trials that have shown that their use as adjuncts to scaling and root planing can result in a significantly greater PD reduction and an increased average CAL gain (mainly in very deep PDs), thus enhancing clinical variable outcomes[84].

Whether or not to utilize local delivery antimicrobials as part of periodontal therapy should be weighed mainly against the strong evidence presented to date in the literature that supports the use of systemic antibiotics as an adjunct to non-surgical periodontal therapy. The dentist should also consider the adverse reactions, as well as the pros and cons, of adjunctive local delivery antimicrobials as an alternative to adjunctive systemic antibiotics. The advantages of local delivery over systemic antibiotics have been described previously, including: 1) better compliance; 2) an enhanced pharmacokinetic response; 3) advantage of placing the active agent in close proximity to a disease site and a lower total dose of drug at a more controlled concentration[122]. Despite the clinical trials supporting the use of local delivery antimicrobials, there are reviews and position papers that state the SRP and the most popular local delivery system today, minocycline microspheres, present only a low level of evidence to support its use with respect to the balance between benefits and adverse effects[86,87]. Despite these recommendations, we do know, as previously mentioned, that not all periodontitis patients should be treated the same with an identical protocol. Each treatment plan should be tailored on a case by case basis and it must be profoundly thought through. Periodontal treatment plans should take

into consideration the overall systemic condition and environmental effects, as well the financial aspects of the treatment plan. The use of local delivery adjuncts can be advocated and should be considered in the proper context.

CONCLUSIONS

Overall, minocycline gel presented clinically a positive response for sites with moderate to severe forms of periodontitis. Minocycline gel as an option to treat multiple sites, with less chairside time and in a more application-friendly manner, makes the gel form a more affordable and thus more accessible method to treat periodontitis patients. In order to see if the results we found were an under - or an overestimation of the true efficacy of the minocycline in a gel form, further and larger scale studies looking into different periodontitis subgroups should be conducted. These subgroups can include studies of multi rooted teeth vs. single rooted teeth, patients with diabetes type 2, tobacco-users, and in clinical cases where esthetics is a concern in order to make more definitive recommendations.

Table 1. Study Timeline and Procedures Completed in the Clinical Trial.

Procedure	Visit 1 Screening	Visit 2 Baseline	Visit 3 Evaluation	Visit 4 Evaluation	Visit 5 Evaluation	Visit 6 evaluation	Visit 7 Evaluation	Visit 8 Evaluation
Day	**-30 to 0**	**Day 0**	**Day 4**	**Day 14**	**Month 1**	**Month 3**	**Month 6**	**Month 9**
Informed consent	**X**							
Medical history	**X**							
Oral examination	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Vital signs		**X**						**X**
Urine pregnancy test (Females)		**X**				**X**	**X**	
SRP		**X**						
Periodontal assessment		**X**			**X**	**X**	**X**	**X**
Treatment administration		**X**		**X**	**X**	**X**	**X**	
Post op OHI		**X**		**X**	**X**	**X**	**X**	**X**
Adverse effect recording		**X**	**X**	**X**	**X**	**X**	**X**	**X**

Table 2. Demographic Data and Tobacco-Use for Enrolled Subjects at the Screening Appointment*.

Variable	Category	Sham control (n=19)	Vehicle control (n=20)	Minocycline gel (n=20)	p-value
Gender n (%)	Male	12 (63%)	12 (60%)	10 (50%)	0.72
	Female	7 (37%)	8 (40%)	10 (50%)	
Age at treatment mean years (SD)		51.5 (13.8)	50.8 (10.5)	53.8 (11.3)	0.71
Race n (%)	Caucasian	15 (79%)	19 (95%)	12 (60%)	0.09
	Black	1 (5%)	0 (0%)	5 (25%)	
	Asian	2 (11%)	1 (5%)	2 (10%)	
	Other	1 (5%)	0 (0%)	1 (5%)	
Tobacco-use n (%)	Non-user	6 (32%)	4 (20%)	8 (40%)	0.74
	Ex-user	6 (32%)	8 (40%)	5 (25%)	
	Current user	7 (37%)	8 (40%)	7 (35%)	
Tobacco amount n (%)	Light	3 (23%)	5 (31%)	3 (25%)	0.60
	Moderate	8 (62%)	8 (50%)	9 (75%)	
	Heavy	2 (15%)	3 (19%)	0 (0%)	
Duration of tobacco-use mean years (SD)		19.8 (14.2)	20.0 (13.9)	22.0 (12.8)	0.90

***** Summaries shown are mean (standard deviation) or n (percent).

Table 3. Mean Clinical Variables for sites evaluated of all Subjects at Baseline in the Three Study Groups*.

Variable	Sham control (n=19)	Vehicle control (n=20)	Minocycline gel (n=19)	p-value
Pocket depth (PD)	5.82 (0.13)	5.65 (0.08)	5.68 (0.09)	0.45
Clinical attachment level (CAL)	5.33 (0.25)	4.79 (0.22)	4.87 (0.26)	0.24
Percent bleeding on probing (BOP)	92.51 (2.57)	95.71 (1.31)	95.46 (1.48)	0.41

* Summaries shown are mean (standard error)

Table 4. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables from Baseline at 3-months from *.

Variable	Sham control (n=19)	Vehicle control (n=20)	Minocycline gel (n=19)	p-value
Pocket depth	4.30 (0.14)	4.15 (0.14)	4.07 (0.14)	0.49
Pocket depth reduction from baseline	1.51 (0.14)	1.50 (0.11)	1.60 (0.14)	0.84
Clinical attachment level	3.85 (0.28)	3.46 (0.25)	3.47 (0.24)	0.48
Clinical attachment gain from baseline	1.48 (0.18)	1.33 (0.11)	1.40 (0.14)	0.77
%BOP	53.77 (5.34)	44.37 (5.99)	37.27 (6.15)	0.15
%BOP reduction from baseline	39.74 (3.96)	51.34 (5.91)	58.19 (5.84)	**0.04****
Percent of sites PDs improved by 2 mm	44.17 (4.75)	50.57 (4.59)	53.23 (4.21)	0.36
Percent of sites PDs improved by 3 mm	19.29 (4.12)	16.73 (3)	17.61 (3.94)	0.88

***** Summaries shown are mean (standard error).

**** Statistically significant (P<0.05)**

Table 5. Mean Clinical Variable for evaluated sites of all Subjects and Changes in Clinical Variables from Baseline at 6-months from *.

Variable	Sham control (n=18)	Vehicle control (n=19)	Minocycline gel (n=19)	p-value
Pocket depth	4.27 (0.17)	4.19 (0.16)	3.99 (0.13)	0.41
Pocket depth reduction from baseline	1.52 (0.14)	1.49 (0.12)	1.69 (0.13)	0.51
Clinical attachment level	3.86 (0.28)	3.48 (0.25)	3.51 (0.26)	0.54
Clinical attachment gain from baseline	1.37 (0.09)	1.31 (0.08)	1.36 (0.12)	0.91
%BOP	41.27 (4.60)	46.06 (4.83)	37.15 (5.44)	0.45
%BOP reduction from baseline	51.24 (3.61)	49.65 (4.72)	58.31 (5.67)	0.38
Percent of sites PDs improved by 2 mm	45.50 (5.40)	48.84 (5.68)	53.73 (4.91)	0.55
Percent of sites PDs improved by 3 mm	16.33 (3.77)	18.03 (2.60)	21.61 (4.14)	0.57

***** Summaries shown are mean (standard error).

Table 6. Mean Clinical Variables for sites evaluated of all Subjects and Changes in Clinical Variables from Baseline at 9-months from*.

Variable	Sham control (n=17)	Vehicle control (n=18)	Minocycline gel (n=19)	p-value
Pocket depth	4.29 (0.18)	3.96 (0.16)	3.91 (0.13)	0.20
Pocket depth reduction from baseline	1.45 (0.13)	1.72 (0.14)	1.76 (0.14)	0.24
Clinical attachment level	3.94 (0.31)	3.18 (0.30)	3.32 (0.26)	0.17
Clinical attachment gain from baseline	1.28 (0.10)	1.56 (0.13)	1.56 (0.12)	0.19
%BOP	40.84 (5.28)	49.63 (4.57)	32.50 (3.71)	**0.03****
%BOP reduction from baseline	51.67 (4.20)	46.08 (4.98)	62.96 (3.94)	**0.03****
Percent of sites PDs improved by 2 mm	45.37 (4.50)	57.95 (5.38)	57.19 (5.21)	0.16
Percent of sites PDs improved by 3 mm	15.32 (3.60)	22.95 (4.45)	22.60 (4.19)	0.35

* Summaries shown are mean (standard error).

**** Statistically significant (P<0.05)**

Table 7. Mean Clinical Variable for Sites Evaluated of all Subjects at baseline for patients with PDs ≥5-≤7 mm*.

Variable	Sham control (n=19)	Vehicle control (n=20)	Minocycline gel (n=19)	p-value
Pocket depth	5.59 (0.08)	5.52 (0.05)	5.52 (0.06)	0.66
Clinical attachment level	5.10 (0.24)	4.68 (0.21)	4.73 (0.24)	0.38
%BOP	93.02 (2.63)	95.92 (1.28)	95.20 (1.55)	0.53

* The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of 5-7 mm at baseline*. Summaries shown are mean (standard error).

Table 8. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables from Baseline at 3-months for patients with PDs ≥5-≤7 mm*.

Variable	Sham control (n=19)	Vehicle control (n=20)	Minocycline gel (n=19)	p-value
Pocket depth	4.19 (0.12)	4.07 (0.14)	3.99 (0.13)	0.54
Pocket depth reduction from baseline	1.40 (0.13)	1.45 (0.11)	1.53 (0.13)	0.77
Clinical attachment level	3.75 (0.29)	3.38 (0.26)	3.38 (0.23)	0.52
Clinical attachment gain from baseline	1.35 (0.15)	1.30 (0.11)	1.36 (0.13)	0.95
%BOP	52.23 (5.45)	43.14 (6.03)	36.86 (6.01)	0.19
%BOP reduction from baseline	52.18 (4.09)	47.29 (5.99)	62.7 (5.71)	0.07
Percent of sites PDs improved by 2 mm	42.46 (4.79)	49.41 (4.64)	51.82 (4.19)	0.33
Percent of sites PDs improved by 3 mm	17.47 (3.97)	16.19 (2.92)	15.30 (3.81)	0.91

***** The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥5-≤7 mm at baseline*. Summaries shown are mean (standard error).

Table 9. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables from Baseline at 6-months for patients with PDs ≥5-≤7 mm *.

Variable	Sham control (n=18)	Vehicle control (n=19)	Minocycline gel (n=19)	p-value
Pocket depth	4.16 (0.14)	4.10 (0.15)	3.90 (0.13)	0.39
Pocket depth reduction from baseline	1.43 (0.13)	1.44 (0.13)	1.62 (0.13)	0.51
Clinical attachment level	3.75 (0.29)	3.40 (0.25)	3.43 (0.26)	0.59
Clinical attachment gain from baseline	1.29 (0.08)	1.29 (0.08)	1.30 (0.13)	0.99
%BOP	39.56 (4.81)	44.48 (4.67)	36.25 (5.55)	0.51
%BOP reduction from baseline	53.46 (3.74)	51.44 (4.45)	58.95 (5.78)	0.49
Percent of sites PDs improved by 2 mm	44.71 (5.33)	46.86 (5.98)	52.67 (4.95)	0.57
Percent of sites PDs improved by 3 mm	15.17 (3.60)	17.36 (2.79)	18.66 (3.99)	0.78

***** The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥5-≤7 mm at baseline*. Summaries shown are mean (standard error).

Table 10. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables from Baseline at 9-months for patients with PDs ≥5-≤7 mm *.

Variable	Sham control (n=17)	Vehicle control (n=18)	Minocycline gel (n=19)	p-value
Pocket depth	4.17 (0.15)	3.86 (0.15)	3.83 (0.12)	0.19
Pocket depth reduction from baseline	1.37 (0.13)	1.69 (0.14)	1.69 (0.14)	0.19
Clinical attachment level	3.84 (0.32)	3.09 (0.30)	3.23 (0.25)	0.17
Clinical attachment gain from baseline	1.21 (0.10)	1.56 (0.13)	1.50 (0.13)	0.10
%BOP	39.39 (5.29)	48.25 (4.69)	32.35 (3.77)	0.06
%BOP reduction from baseline	53.63 (4.09)	47.67 (5.12)	62.85 (4.06)	0.06
Percent of sites PDs improved by 2 mm	44.17 (4.53)	57.17 (5.34)	55.66 (5.25)	0.16
Percent of sites PDs improved by 3 mm	14.40 (3.48)	22.70 (4.54)	20.25 (4.16)	0.36

* The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥5-≤7 mm at baseline*. Summaries shown are mean (standard error).

Table 11. Demographics and Tobacco-Use for patients in the three study groups with PDs ≥8 mm at baseline*.

Variable	Category	Sham control (n=10)	Vehicle control (n=10)	Minocycline gel (n=13)	p-value
Gender n (%)	Male	7 (70%)	7 (70%)	7 (54%)	0.73
	Female	3 (30%)	3 (30%)	6 (46%)	
Age at treatment mean years (SD)		51.6 (11.8)	51.9 (12.3)	52.6 (10.1)	0.98
Race n (%)	Caucasian	9 (90%)	9 (90%)	6 (46%)	0.12
	Black	1 (10%)	0 (0%)	4 (31%)	
	Asian	0 (0%)	1 (10%)	2 (15%)	
	Other	0 (0%)	0 (0%)	1 (8%)	
Tobacco-use n (%)	Non-user	3 (30%)	3 (30%)	5 (38%)	>0.99
	Ex-user	3 (30%)	3 (30%)	3 (23%)	
	Current user	4 (40%)	4 (40%)	5 (38%)	
Duration of tobacco-use mean years (SD)		16.9 (11.8)	24.1 (14.1)	21.4 (13.3)	0.59

* Summaries shown are mean (standard deviation) or n (percent).

Table 12. Mean Clinical Variables for sites evaluated of all Subjects with PDs ≥8 mm at Baseline*.

Variable	Sham control (N=10)	Vehicle control (N=10)	Minocycline gel (N=13)	P-value
Pocket depth	8.38 (0.13)	8.09 (0.05)	8.15 (0.08)	0.08
Clinical attachment level	8.06 (0.27)	6.71 (0.45)	7.02 (0.36)	**0.05****
%BOP	86.25 (6.57)	89.17 (5.83)	100 (0.00)	0.09

***** The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥8 mm at baseline*. Summaries shown are mean (standard error). For variables having significant group differences, p-values from post-hoc tests for pairwise group comparisons are shown in Table 17.

**** Statistically significant (P<0.05)**

Table 13. Mean Clinical Variables for Sites Evaluated of all Subjects and Changes in Clinical Variables at 3-Months from Baseline for Patients with PDs ≥8 mm*.

Variable	Sham control (n=9)	Vehicle control (n=10)	Minocycline gel (n=13)	p-value
Pocket depth	5.68 (0.51)	5.88 (0.51)	5.28 (0.37)	0.62
Pocket depth reduction from baseline)	2.64 (0.47)	2.21 (0.53)	2.87 (0.39)	0.58
Clinical attachment level	5.48 (0.41)	5.02 (0.70)	4.96 (0.47)	0.77
Clinical attachment gain from baseline	2.41 (0.53)	1.69 (0.53)	2.07 (0.36)	0.58
%BOP	58.87 (12.36)	66.67 (12.91)	54.12 (11.56)	0.76
%BOP reduction from baseline	27.38 (14.13)	22.50 (10.83)	45.88 (11.56)	0.37
Percent of sites PDs improved by 2 mm	63.25 (11.85)	57.74 (13.57)	77.75 (8.73)	0.41
Percent of sites PDs improved by 3 mm	47.46 (10.97)	25.71 (11.38)	54.12 (10.85)	0.19

***** The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥8 mm at baseline*. Summaries shown are mean (standard error).

Table 14. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables at 6-Months from Baseline for Patients with PDs ≥8 mm *.

Variable	Sham control (n=8)	Vehicle control (n=10)	Minocycline gel (n=13)	p-value
Pocket depth	5.74 (0.63)	6.15 (0.32)	5.24 (0.34)	0.28
Pocket depth reduction from baseline	2.61 (0.56)	1.94 (0.35)	2.91 (0.28)	0.17
Clinical attachment level	5.39 (0.54)	5.08 (0.46)	4.65 (0.43)	0.54
Clinical attachment gain from baseline	2.32 (0.47)	1.63 (0.39)	2.38 (0.27)	0.28
%BOP	47.46 (13.98)	72.14 (10.83)	40.93 (9.68)	0.12
%BOP reduction from baseline	38.79 (17.66)	17.03 (11.62)	59.07 (9.68)	0.06
Percent of sites PDs improved by 2 mm	69.32 (12.56)	64.40 (12.95)	76.65 (8.72)	0.71
Percent of sites PDs improved by 3 mm	40.07 (14.14)	30.36 (11.66)	71.70 (8.79)	**0.02****

***** The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥8 mm at baseline*. Summaries shown are mean (standard error).

**** Statistically significant (p<0.05)**

Table 15. Mean Clinical Variable for Sites Evaluated of all Subjects and Changes in Clinical Variables at 9-Months from Baseline for Patients with PDs ≥8 mm *.

Variable	Sham control (n=7)	Vehicle control (n=9)	Minocycline gel (n=13)	p-value
Pocket depth	5.99 (0.45)	6.19 (0.32)	4.87 (0.35)	**0.03****
Pocket depth reduction from baseline	2.34 (0.41)	1.88 (0.34)	3.27 (0.36)	**0.03****
Clinical attachment level	5.60 (0.38)	5.11 (0.59)	4.31 (0.56)	0.28
Clinical attachment gain from baseline	2.18 (0.37)	1.35 (0.32)	2.71 (0.30)	**0.01****
%BOP	73.95 (8.23)	68.78 (13.77)	36.81 (9.62)	**0.04****
%BOP reduction from baseline,	12.30 (8.22)	20.39 (11.47)	63.19 (9.62)	**0.05****
Percent of sites PDs improved by 2 mm	75.13 (11.06)	59.79 (13.11)	89.29 (6.62)	0.10
Percent of sites PDs improved by 3 mm	33.80 (14.19)	21.43 (8.94)	62.91 (10.89)	**0.03****

* The data was aggregated at the patient level by averaging across the outcomes for all sites with a *pocket depth of ≥8 mm at baseline*. Summaries shown are mean (standard error).

** **Statistically significant (P<0.05)**

Table 16. p-values for Intragroup analysis for statistically significant findings of the Various Clinical Variables and Clinical Variables Change*.

Variable	Group	Timing	Sham Control vs. vehicle control	Minocycline gel vs. Sham Control	Minocycline gel vs. Vehicle control
Percent bleeding on probing reduction from baseline	All patients	3 months	0.22	**0.03****	0.64
%BOP	All patients	9 months	0.37	0.40	**0.02****
%BOP reduction from baseline	All patients	9 months	0.61	0.21	**0.02****
Clinical attachment level	Sites of ≥8 mm	Baseline	0.06	0.13	0.82
Percent of sites improved by 2 mm	Sites of ≥8 mm	6 months	0.83	0.14	**0.02****
Pocket depth	Sites of ≥8 mm	9 months	0.94	0.12	**0.03****
Pocket depth reduction from baseline	Sites of ≥8 mm	9 months	0.72	0.23	**0.03****
Clinical attachment gain from baseline	Sites of ≥8 mm	9 months	0.26	0.51	**0.01****
Percent bleeding on probing	Sites of ≥8 mm	9 months	0.95	0.07	0.10
Percent bleeding on probing reduction from baseline	Sites of ≥8 mm	9 months	0.95	**0.01****	**0.01****
Percent of sites improved by 3 mm	Sites of ≥8 mm	9 months	0.77	0.21	**0.03****

* p-values from post -hoc tests for pairwise group comparisons for variables for which the overall group comparisons were statistically significant.

** **Statistically significant (P<0.05)**

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