Summer 8-13-2021

The Effect of Locally Applied Simvastatin on Clinical Attachment Level and Alveolar Bone in Periodontal Maintenance Patients

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THE EFFECT OF LOCALLY APPLIED SIMVASTATIN ON CLINICAL ATTACHMENT LEVEL AND ALVEOLAR BONE IN PERIODONTAL MAINTENANCE PATIENTS

by

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A THESIS

Presented to the Faculty of

The University of Nebraska Graduate College

In Partial Fulfillment of the requirements

For the Degree of Master of Science

Medical Sciences Interdepartmental Area
Oral Biology

Under the Supervision of Professor Amy C. Killeen

University of Nebraska Medical Center

Omaha, Nebraska

June 2021

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Acknowledgements

As I reflect on my academic journey that has brought me to this point, I am filled with much gratitude for the many opportunities I have been given, I am thankful for all the people that have helped me along the way, and I am reminded of the countless hours and determination it has taken me to complete my certificate in periodontics and Master's of Science in Oral Biology.

Firstly, I would like to thank my parents, Greg and Lynn Krell, for always giving me the confidence and support to pursue my dreams. Throughout every stage in my academic journey, I have always known I have two cheerleaders that not only were on my side, but would sacrifice to help me in any way they could. A special thank you to Benjamin Coenen for his unwavering love and encouragement and to my son, Tripp, for motivating me to work hard in everything I do.

I would like to thank my advisor, Dr. Amy Killeen, for her guidance in this process. Her thoroughness and commitment to organize the required protocols were critical in gaining IRB approval for the trial and for the study to run as smoothly as possible. It has been extremely rewarding for me to learn from and be supported by a female periodontist that as a dental student I always looked up to as an example of what I someday hoped to become.

I would also like to thank Dr. Richard Reinhardt for his preceding work in simvastatin research in periodontitis, for his very active role in my project, and for trusting me to be one of the surgeons treating the patients of this study. Over the past three years of my involvement in this study, Dr. Reinhardt was always willing to discuss any aspect of this trial and help answer my questions. I am honored to have had the opportunity to learn from a leading expert in the field of periodontics.

Additionally, I would like to thank the other members of my research committee. Dr. Jeffrey Payne for never hesitating to take the time to share his extensive knowledge of periodontics with me and for his enthusiasm in teaching periodontics. Dr. Greg Oakley and Dr. Jim Wahl for their thoughtful advice and insight during the progression of this study. Megan Christiansen and Laura Anderson for their essential roles in organizing the recruitment of patients, setting up and assisting each baseline appointment, and performing the periodontal maintenance procedures for the twelve months of the study. I would also like to thank Kaeli Samson for providing the statistical analysis work and expertise in her area.

Finally, thank you to my co-resident, Dr. Devin Christiansen, for all of the fun times mixed in with a lot of hard work over the last three years!

THE EFFECT OF LOCALLY APPLIED SIMVASTATIN ON CLINICAL ATTACHMENT LEVEL AND ALVEOLAR BONE IN PERIODONTAL MAINTENANCE PATIENTS

Lauren E. Krell, D.D.S., M.S.

The University of Nebraska Medical Center, 2021

Advisor: Amy Killeen, D.D.S, M.S.

The purpose of this double-blinded, randomized, controlled trial was to determine if the local application of simvastatin (SIM), combined with minimally invasive papilla reflection and root preparation (PR/RP), is effective in improving clinical attachment level (CAL), primary outcome measure; probing depth (PD) reduction; increasing interproximal bone height (IBH); and reducing bleeding on probing (BOP) in persistent 6-9 mm periodontal pockets in patients receiving periodontal maintenance therapy (PMT). Fifty patients with Stage III, Grade B periodontitis (AAP, 2018) presenting with a 6-9 mm interproximal PD with a history of bleeding on probing (BOP) were included in the study. Experimental 2.2 mg simvastatin in 0.15 ml methylcellulose (PR/RP+SIM/MCL; n=27) and methylcellulose alone (MCL) control (PR/RP + MCL; n=23) therapies were randomly assigned. Root surfaces were accessed via reflection of interproximal papillae, followed by root planing assisted with endoscope evaluation, acid etching, and SIM/MCL or MCL application. Clinical measurements were collected at baseline and 12 months. IBH measurements were assessed on standardized vertical bitewing radiographs taken at baseline and 12 months. Both PR/RP+SIM/MCL and PR/RP+MCL resulted in improvements in (CAL: -1.93 ± 0.31 mm, $p<0.0001$; -1.01 ± 0.32 mm, $p<0.003$; PD: -2.34 mm ± 0.26, $p<0.0001$; -1.34 mm ± 0.28, $p<0.0001$) and stable IBH (-0.17 ± 0.15, -0.42 ± 0.16, $p=0.22$) from baseline to 12 months post-therapy. The addition of SIM/MCL to PR/RP improved CAL and PD compared to MCL alone in periodontal maintenance patients.

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LIST OF ABBREVIATIONS

AAP	American Academy of Periodontology
BOP	bleeding on probing
CAL	clinical attachment level
CEJ	cementoenamel junction
EDTA	ethylenediaminetetraacetic acid
GCF	gingival crevicular fluid
IBH	interproximal bone height
PD	probing depth
PDL	periodontal ligament
PL	plaque
PI	plaque index
PID	positioning indicating device
PMT	periodontal maintenance therapy
PR/RP + SIM/MCL	papilla reflection with root planing and simvastatin/methylcellulose application (test)
PR/RP + MCL	papilla reflection with root planing and methylcellulose application (control)
REC	gingival recession
SIM	simvastatin
SRP	scaling and root planning

CHAPTER 1: Introduction

Periodontitis is an inflammatory disease that results in loss of alveolar bone, gingival recession, and periodontal pocketing due to the degradation of the periodontium. The resultant bone resorption causes loss of periodontal attachment and can lead to tooth loss. Periodontal disease impacts much of the United States population, affecting approximately 46 percent of those 30 years of age or older, with males and those who smoke being at greater risk (Eke et al., 2016).

The detection and diagnosis of periodontitis is determined via clinical measurements and radiographic evidence of bone loss acquired during periodontal examination. For the clinician to arrive at a diagnosis of periodontitis, the following parameters can be assessed: presence of clinical signs of inflammation (bleeding, edema, erythema of soft tissues); probing depths; medical and dental histories; and signs and symptoms, including pain, ulceration, and amount of detectable plaque and calculus (AAP Position Paper, 2003). Disease severity and extent is classified by assigning a stage and grade of disease that helps to identify patients' complexity of management and risk of progression (Tonetti et al., 2018). In summary of the periodontal disease classification, patients are assigned a numerical stage (I-IV), which is dependent upon the severity and complexity of disease, and also assigned a grade (A-C), which is intended to assess the likelihood of the disease progressing at a greater rate than normally expected or responding less predictably to therapy.

Traditional protocols for periodontal therapy are centered on subgingival debridement by means of scaling and root planing to control subgingival microflora and contaminated root surface known to drive destruction of the periodontium (Cobb et al., 2002). It has been shown that when comparing pocket depth, root planing by means of closed debridement is less

effective in calculus removal the deeper the pocket (Caffesse et al., 1986) and could result in residual pocketing. The residual deeper periodontal pockets have an increased incidence for future attachment loss (Kaldahl et al., 1996). To ensure more calculus removal and better access at the depth of the pocket, other treatment approaches, such as open flap debridement or a minimally invasive flap access to root surfaces, may need to be performed in combination with or following nonsurgical therapy. Open flap debridement is performed via an incision at the gingival margin, followed by a full thickness gingival flap elevation, allowing visualization of the root surfaces and osseous architecture. Combining open flap debridement with scaling and root planing is more effective than closed flap scaling and root planing regardless of clinician experience level (Brayer et al., 1989). However, there can be some disadvantages to open flap debridement, such as recession or sensitivity. Furthermore, not all patients are accepting of invasive periodontal surgical procedures. A papilla reflection approach differs from open flap debridement in that it is more conservative and a single interdental papilla is reflected instead of a longer span flap that extends across multiple teeth. Less invasive papilla reflection access has also been shown to improve calculus removal (Johnson et al., 1989). In efforts to eliminate residual pockets, surgical access via a minimally invasive flap combined with the use of an endoscope is another approach that can aid to increase thoroughness of instrumentation, potentially improving outcomes of therapy and increasing probing depth reduction. It has been shown that the use of an endoscope with scaling and root planning removes significantly more calculus versus scaling and root planning alone (Geisinger et al., 2007).

Once a patient's periodontitis is deemed stable, a periodontal maintenance recall is implemented, usually every three to four months in frequency to assist in maintaining a patient's periodontal health. Periodontal maintenance includes removal of bacterial plaque and calculus from supragingival and subgingival regions via mechanical instrumentation, selective

root planing if indicated, polishing of teeth, and a review of patient's plaque removal efficacy (AAP Position Paper, 2003). Good compliance with periodontal maintenance recall is important for long-term tooth retention. Studies have shown that patients undergoing regular periodontal maintenance therapy have less incidence of periodontal breakdown and keep their teeth longer than those who are erratic or non-compliers (Wilson et al., 1987; Costa et al., 2011).

Not all patients or sites respond equally to traditional periodontal maintenance therapy (PMT). To address residual inflamed or progressing pockets during PMT, adjunctive therapies have been developed to further reduce bacterial loads, inflammation, PD and CAL. Such adjuncts include local delivery of anti-inflammatory or anti-microbial agents. Simvastatin is a specific competitive inhibitor of 3-hydroxy-2-methylglutaryl coenzyme A reductase and was originally used to reduce serum cholesterol, yet also has been shown to have anti-inflammatory and bone anabolic properties (Mundy et al., 1999). The effectiveness of simvastatin in the treatment of periodontitis has been shown in animal and human studies as an adjunct to initial scaling and root planing treatment in periodontal disease. It has never before been shown in a human periodontal maintenance application. Local application of statins has been shown to reduce periodontal pocket probing depths, reduce clinical attachment loss, and reduce inflammation in human clinical trials outside the United States when incorporated in scaling and root planing, or initial therapy (Pradeep, 2010). Further research is needed to explore the effects of simvastatin when applied to residual pockets in patients that have already undergone initial therapy and are receiving PMT.

The hypothesis of this study is that local application of simvastatin (SIM) in a methylcelluslose carrier (MCL) via surgical papilla reflection, root planing and endoscopic evaluation in persistent 6-9 mm pockets is effective in decreasing clinical attachment

loss (primary outcome), as well as reducing PD, BOP and increasing bone height compared to MCL alone in patients on PMT.

CHAPTER 2: Literature Review - Periodontitis

Periodontitis is an inflammatory disease that results in the destruction of the alveolar bone causing attachment loss and periodontal pocketing. Clinically, periodontal disease presents with gingival erythema, deepened periodontal probing depth, loss of clinical attachment level (CAL), tooth mobility, bleeding, suppuration, and gingival recession.

Although the primary cause of periodontal destruction is bacterial plaque, the etiology is multifactorial with a known interaction between oral microbes and the host immune response (Socransky et al., 1992). It is known that certain pathogens are required but not sufficient for periodontal disease activity to occur and that the host must have susceptibility for progression (Preshaw, 2008). Twin studies have been conducted that are suggestive of genetic inheritance attributing to disease progression (Michalowicz et al., 1991). Some specific genetic conditions, including neutrophil dysfunction and IL-1 polymorphism, have also been suggested as being contributory to various forms of periodontitis (Kornman et al., 1997; Van Dyke et al., 1985).

If periodontitis is not treated, the result can be continued attachment loss leading to eventual tooth loss (AAP Position Paper, 2003). Treatment can consist of non-surgical therapy, surgical therapy, or a combination of both. Non-surgical periodontal therapy, or scaling and root planing (SRP), has been coined the “gold standard” of periodontal treatment (Cobb et al., 2002). The instrumentation of root surfaces as part of SRP for the removal of the biofilm can be accomplished via hand or ultrasonic instruments. Studies have shown that disease progression is halted by inflammation reduction and restoring the patient to a comfortable and functional dentition (Zander et al., 1976).

To evaluate the results of SRP, clinical measurements are compared to baseline and the improvement is measured by the reduction in PD, CAL gain, decrease in BOP, and plaque index (Haffajee et al., 1997). Following positive response to scaling and root planning therapy, a periodontal maintenance therapy (PMT) at 3-4 monthly intervals is implemented. Periodontal maintenance consists of extraoral and intraoral examination, periodontal evaluation, utilizing instruments to remove bacterial plaque and calculus from supragingival and subgingival tooth surfaces, selective root planing, radiographic review, and a review of patient's plaque removal efficacy (AAP Position Paper 2003). The goals of PMT are to prevent or minimize the recurrence of disease progression, reduce the incidence of tooth loss, and increase the probability of locating and treating other conditions or diseases found within the oral cavity (Cohen et al., 2003). Patients who receive periodontal therapy and do not follow through with maintenance do not see long-term improvements in PD and bone levels. In these patients, the improvements seen following periodontal therapy are lost, and they eventually return to a diseased state (Axelsson et al., 1981).

If following initial nonsurgical therapy, signs of disease activity persist (bleeding on probing, increased periodontal pocketing, attachment loss, bone loss), then additional therapies such as surgical intervention, pharmacotherapeutics, or biomaterials can be considered. Continued therapy for non-responding sites is crucial as sites that have residual signs of inflammation and residual deep PDs are more likely to progress and experience recurrence of disease (Renvert et al., 2002).

CHAPTER 3: Literature Review - Endoscope

The primary objective of instrumentation performed in periodontal therapy is to remove all bacterial plaque and calculus from the root surface. However, despite a clinician's best efforts and perceived smooth root surfaces, many studies have shown the inefficiency of instrumentation in the complete removal of subgingival deposits (Jones et al., 1972). With increased depth of pocket, a decreased likelihood of achieving a calculus free root surface results. Curette efficacy ranges from 2-4 mm, with a calculus free surface only being found up to 3.73 mm in depth (Stambaugh et al., 1981). Residual calculus can negatively impact the healing response of pockets and result in continued inflammation. A high correlation exists between residual calculus and deeper probing depths (Rabbani et al., 1981).

In order to properly instrument a root surface, it is important for clinicians to be able to accurately detect the presence or absence of calculus. Unfortunately, studies have shown that the accuracy of a clinician's subgingival calculus detection is 75% and the accuracy of a clinician to detect a calculus free root surface is 50% (Sherman et al., 1990). Direct visualization can aid in detection accuracy when the clinician is not relying solely on tactile detection. For example, when microscopic visualization is used, greater accuracy is seen in calculus identification when compared to clinical tactile detection alone (Sherman et al., 1990). The dental endoscope was developed with the intention of providing imaging below the marginal gingiva for diagnosis and as an aid in treatment of periodontal disease (Stambaugh et al, 2002). An endoscope allows for both real-time visualization and magnification that can aid in detecting calculus. Other anatomical features can also be visualized such as root fractures, root caries, and open restorative margins may otherwise go undetected. To aid in visualization, dental endoscopes use a fiber-optic cord inserted into the sheath that contain a light source for illumination. The images captured by the endoscope are magnified and displayed on a screen attached to the unit

to allow for real-time visualization. Additionally, simultaneous water flow serves to clear the biofilm, calculus, blood, and other debris that can impede the clinician's view.

The endoscope is designed so that it can be used in conjunction with hand or ultrasonic instruments. Its use can potentially increase the detection and the removal of deposits. A recent systematic review found that with the help of an endoscope significantly more calculus was removed from sites compared to sites scaled and root planed without the aid of the endoscope (Kuang et al., 2017). The addition of an endoscope may or may not result in an advantage in clinical parameters compared to instrumentation alone. A recent clinical trial compared clinical parameters following root surface debridement with and without periodontal endoscopy and found significantly fewer deep probing depths (7-9mm) at three and twelve months when endoscopic visualization was used (Naicker et al, 2021). Due to the potential for increased detection and removal of subgingival debris, the use of a periodontal endoscope is a minimally invasive adjunct that can easily be implemented into therapy.

CHAPTER 4: Literature Review - Simvastatin

Simvastatin (SIM) is a specific competitive inhibitor of 3-hydroxy-2-methylglutaryl coenzyme A reductase that is commercially available, FDA-approved, and commonly taken systemically to regulate cholesterol. Mevalonate is the product of HMG-CoA reductase and is the precursor of many other non-steroidal isoprenoid compounds besides cholesterol and, therefore, has effects other than cholesterol reduction (Sirtori et al., 2014). Anti-inflammatory, immunomodulatory and antimicrobial properties are additional effects produced by statins (Stancu et al., 2001). It has also been shown that bone turnover and regeneration is influenced by statins' action on osteoblasts, osteoclasts, endothelial cells and mesenchymal stem cells. (Edwards et al., 2002).

Statin drugs have been shown to be anti-inflammatory, are low cost and have a high safety profile, therefore making them ideal for potential application in periodontitis treatment for inflammatory reduction and bone regeneration. In 2002, a single high dose local application of simvastatin gel was shown to stimulate murine calvarial bone growth (Thylin et al., 2002). Due to resultant tissue inflammation when delivered at a high dose, simvastatin was investigated at a lower topical dose, which resulted in reduced inflammation without sacrificing bone-growth potential (Stein et al., 2005). Following the reduced inflammatory reaction and bone growth seen at a lower topical dose, simvastatin was studied in an application that more closely mimicked a periodontal defect, a bony dehiscence defect in dogs. In 2011, local application of SIM was placed in dehiscence defects adjacent to the roots of teeth in beagle dogs and resulted in bone induction (Rutledge et al., 2011). To further explore the anti-inflammatory and anabolic properties of SIM, subsequent studies were conducted in animal models with a SIM prodrug application in defects or a combination of SIM and antiresorptive agents, all of which showed greater anti-inflammatory effects and/or bone anabolic effects

when compared to controls (Killeen et al, 2012; George et al., 2013; Price et al, 2013; Bradley et al., 2016).

SIM has been studied to assess its effects in areas of attachment loss resulting from periodontitis in humans but has only been investigated as part of scaling and root planing therapy. When applied locally to defects as part of initial nonsurgical therapy in patients diagnosed with aggressive periodontitis, SIM was found to increase clinical attachment level and bony defect fill versus scaling and root planning alone (Priyanka et al., 2017). Another investigation of SIM application included patients diagnosed with chronic periodontitis and found greater reduction in probing depths, gingival bleeding, and a gain in defect fill when SIM was applied at the time of scaling and root planing (Pradeep et al., 2010).

In addition to SIM, other statins have been found to be effective in the treatment of periodontal defects. Specifically, in a recent systematic review rosuvastatin and atorvastatin resulted in additional reduction of PD when applied locally to mechanical therapy; however, SIM was the only drug that produced significant reduction in CAL (Muniz et al., 2018). Another recent systematic review included 10 studies utilizing statins (simvastatin, atorvastatin and rosuvastatin) during SRP found that local delivery resulted in additional reduction of pocket depth and clinical attachment gain in healthy people, smokers and diabetic patients (Ambrosio et al., 2018).

A gap in knowledge exists in identifying the most clinically applicable use of SIM in patients with periodontal disease. Locally applied SIM has been investigated for purposes of inflammatory reduction and bone regeneration; however, the studies to date have been completed in animals or in humans as part of initial scaling and root planing therapy. This study is the first human clinical trial using locally applied SIM during periodontal maintenance therapy.

CHAPTER 5: Methods

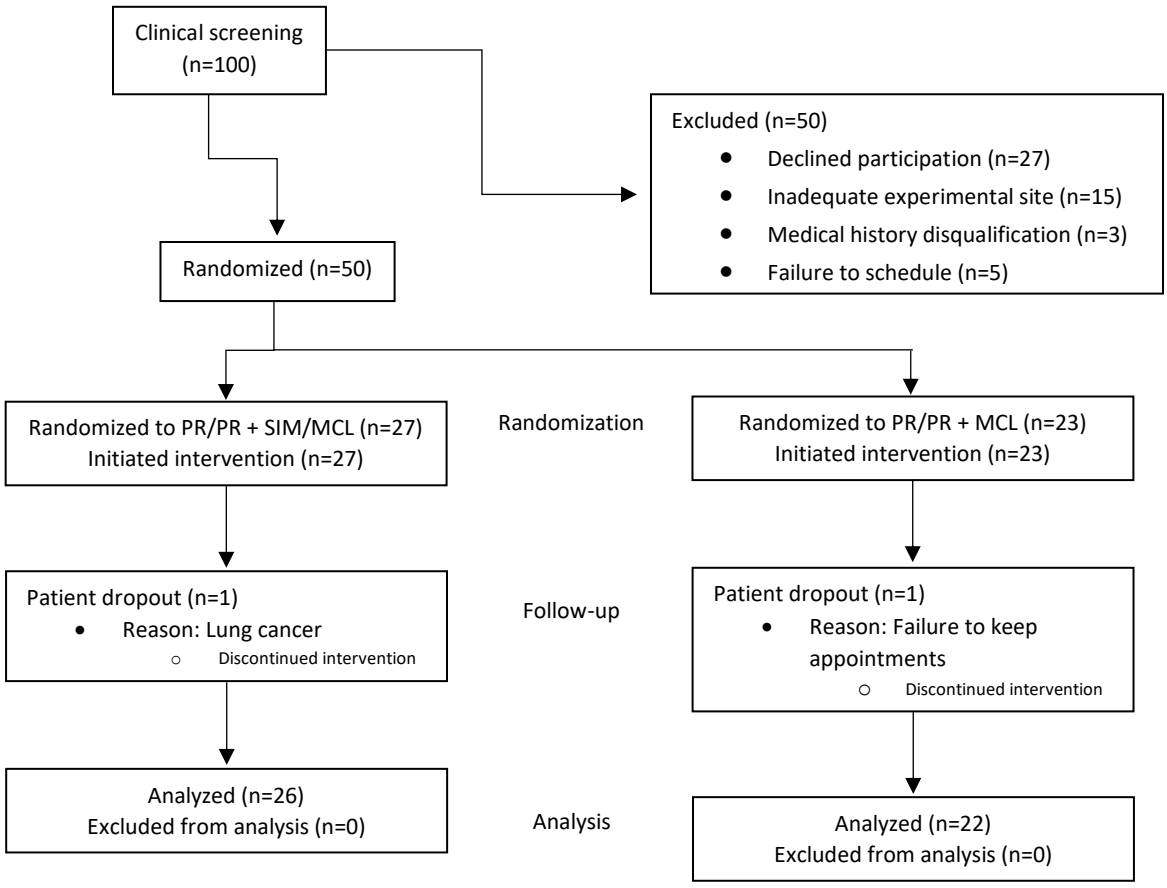
Study Population and Research Design

This 12 month, randomized, double-masked, parallel interventional, clinical trial included randomization of 50 patients who were undergoing periodontal maintenance therapy at the University of Nebraska Medical Center (UNMC) College of Dentistry in Lincoln, Nebraska or in private practices in Grand Island and Lincoln, Nebraska. The flow of study design is included in Figure 1. The following inclusion criteria were used: 1) 40-85 years of age; 2) diagnosis of Stage IV, Grade B periodontitis (AAP, 2018); 3) with at least one interproximal 6-9 mm periodontal probing depth with history of bleeding on probing; 4) overall good systemic health; 5) history of routine periodontal maintenance therapy; and 6) signed consent to participate in this 12-month study. Exclusion criteria were: 1) systemic diseases which significantly impact periodontal inflammation and bone turnover (e.g., rheumatoid arthritis); 2) taking drugs which significantly impact periodontal inflammation and bone turnover (e.g., chronic use of steroids or non-steroidal anti-inflammatory drugs (e.g. >325 mg/d aspirin), estrogen, bisphosphonates, calcitonin, methotrexate, antibiotics); 3) periodontal surgery within the past 1 year; or 4) pregnant or breastfeeding feeding females. Patients taking simvastatin or other HMG-CoA reductase inhibitors systemically were not excluded from the study due to the local application of the drug in this protocol.

The protocol was approved by the University of Nebraska Medical Center Institutional Review Board, Omaha, Nebraska (protocol #217-18-FB) and was in accordance with the Declaration of Helsinki of 1975, as revised in 2013. The clinical study was performed from January 2019 to August 2020 and was registered with ClinicalTrials.gov as NCT03452891.

Periodontal maintenance patients who were screened and met inclusion criteria were randomized into one of two groups for treatment of the 6-9 mm interproximal pocket: 1) papilla reflection and root planing with endoscopic visual verification of a clean surface, root etching with ethylenediaminetetraacetic acid (EDTA) for two minutes, injection with 2.2 mg simvastatin in 0.15 ml methylcellulose gel (PR/RP + SIM/MCL) 2) control with same treatment sequence except injection of methylcellulose gel without simvastatin (PR/RP + MCL). The surgical protocol and study desing was modeled after a recent clinical trial utilizing PR/RP + Emdogain local application (Jasa et al, 2020).

Figure 1: Flow of Study Design

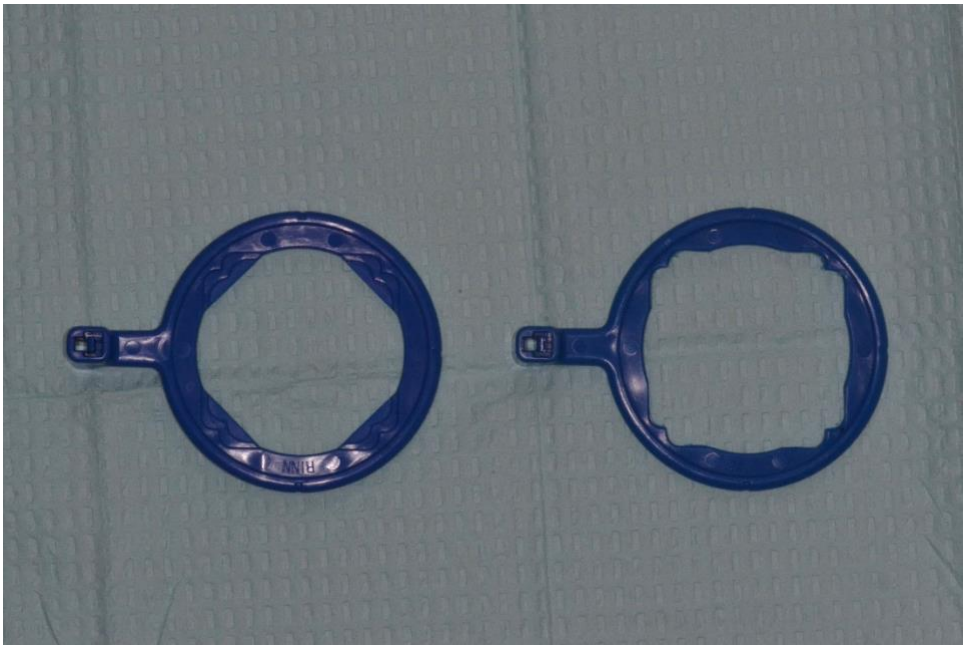


Sample Collection and Clinical Measurements

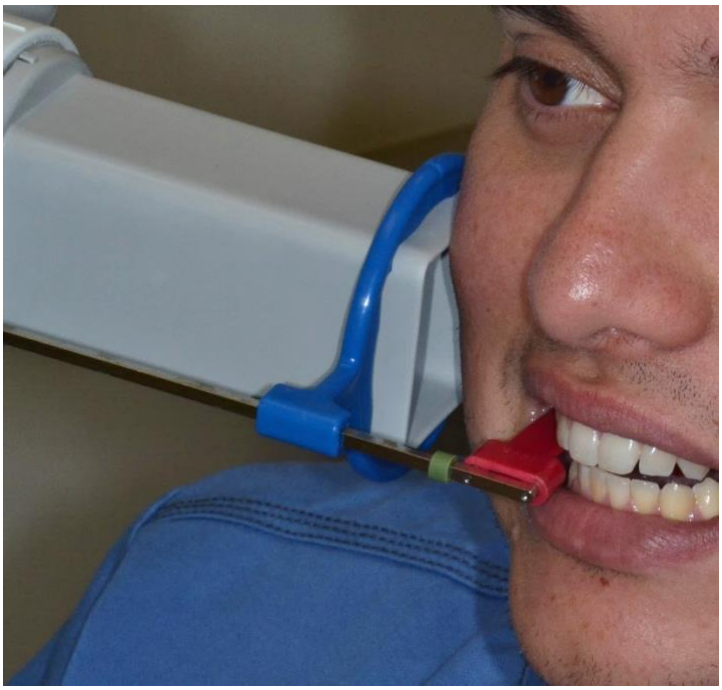
Three examiners were calibrated for reproducibility using 36 maxillary and mandibular posterior sites in a Stage III, Grade B patients with 6-9 mm pockets for PD and CAL measurements within ± 1 mm (RH-RR=91, 83%, RH-AK=83,83%, AK-RR=89, 100%). These masked examiners obtained baseline, two week, and 12-month measurements for gingival recession and probing depths of experimental and adjacent tooth, and clinical attachment levels. Supragingival plaque (PL) was recorded at baseline as either present or absent upon explorer (Gracey 11/12) detection at six sites on the experimental tooth and adjacent tooth. Gingival recession (REC) was measured with a periodontal probe (UNC 15) from cementoenamel junction (CEJ) to the free gingival margin at six sites on each experimental and adjacent tooth. For teeth with restorations obstructing the anatomical CEJ, the most apical extent of the restoration was used as the point of reference for measurements. Probing depths (PD) were measured with a periodontal probe (UNC 15) from the free gingival margin until resistance was felt at the base of the pocket for six sites on experimental and the adjacent tooth. The six sites for which recession, probing depths, and plaque presence were measured were mesial-facial, mid-facial, distal-facial, mesial-lingual, mid-lingual, and distal-lingual. Clinical attachment level was measured by the addition of REC and PD.

Vertical bitewings were taken of each experimental site with the use of a positioning indicating device (PID) at baseline and at the conclusion of the study (Figure 2). Baseline and 12-month measurements were taken for the two proximal tooth surfaces including the experimental site and the adjacent tooth from CEJ to the most coronal aspect of the alveolar crest (Figure 3). If the presence of a restoration obstructed the anatomical CEJ, the most apical extent of the restoration was used for all measurements.

Figure 2: Radiographic PID Aligner

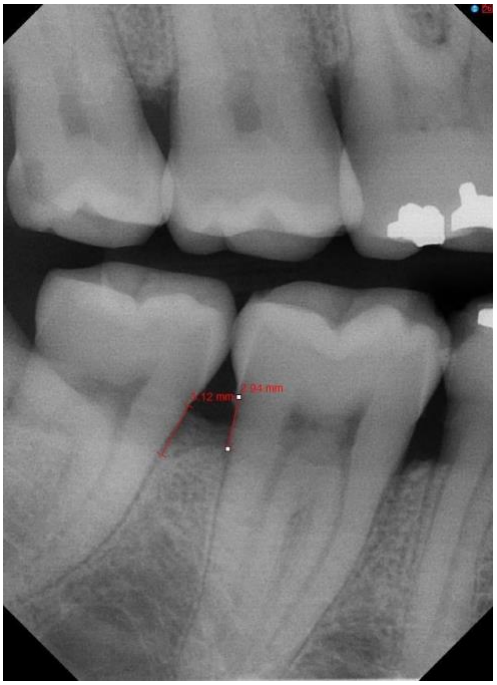


Left: Standard PID aligner Right: Modified PID aligner (used in this study)

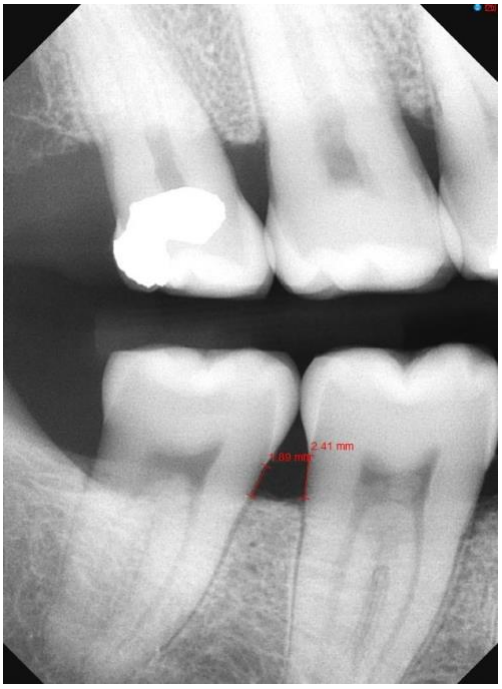


Modified PID aligner in use

Figure 3: Radiographic Bone Height Measurements



Baseline Radiograph



12–month Radiograph

Treatment

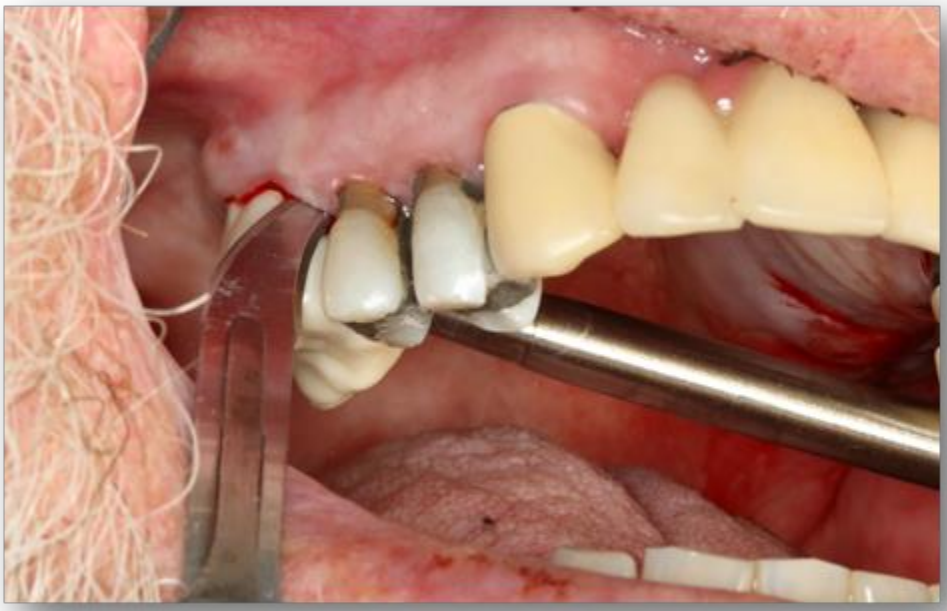
Surgical protocols on the 50 enrolled patients were completed by one of two periodontal residents (MB, LK) (Figure 4). At baseline, a vertical bitewing of the experimental site was obtained utilizing the standardizing PID. Local anesthetic was administered via infiltration of the surgical site. A 12 blade was used for buccal and lingual sulcular incisions from line angle of the experimental tooth to the adjacent tooth proximal line angle. Across the papilla, an inverse bevel technique was used to spare the papilla and allow for removal of proximal col tissue for visualization and osseous crest access. The proximal col tissue was removed with a universal curette following flap reflection with a periosteal elevator. The experimental and adjacent tooth proximal surfaces were scaled and root planed with the posterior universal curette and an ultrasonic instrument (Cavitron, Dentsply, York, PA) for removal of supragingival and subgingival calculus and plaque. Verification of thorough debridement was completed using an endoscope with fiberoptic visualization (Perioscope, Zest Dental Solutions, San Ramon, CA) and an explorer (Hu-Friedy #11/12). Repeated planing was performed until the proximal surfaces were free of calculus and plaque contamination. Normal saline irrigation was performed after instrumentation, followed by application of EDTA (Pref-Gel, Straumann, Andover, MA) for two minutes with a final saline irrigation. After these steps of the procedure, the surgeon accessed the randomization chart to determine if the patient was in the test or control group. Simvastatin and methylcellulose were formulated by a local formulating pharmacy (Pharmacy Solutions, Lincoln, NE) with approval of the UNMC Pharmacy & Therapeutics Committee and IRB. Simvastatin powder and methylcellulose gel were prepared in separate syringes in a certified sterile room. Lots were tested at UNMC Microbiology Clinical Laboratory (Omaha, NE) and were shown to have no bacterial contamination growth of aerobes

or anaerobes, and potency was 96.9% at 37 days after formulation (Eagle Science-Based Solutions, Houston, TX). Preparations were reformulated monthly.

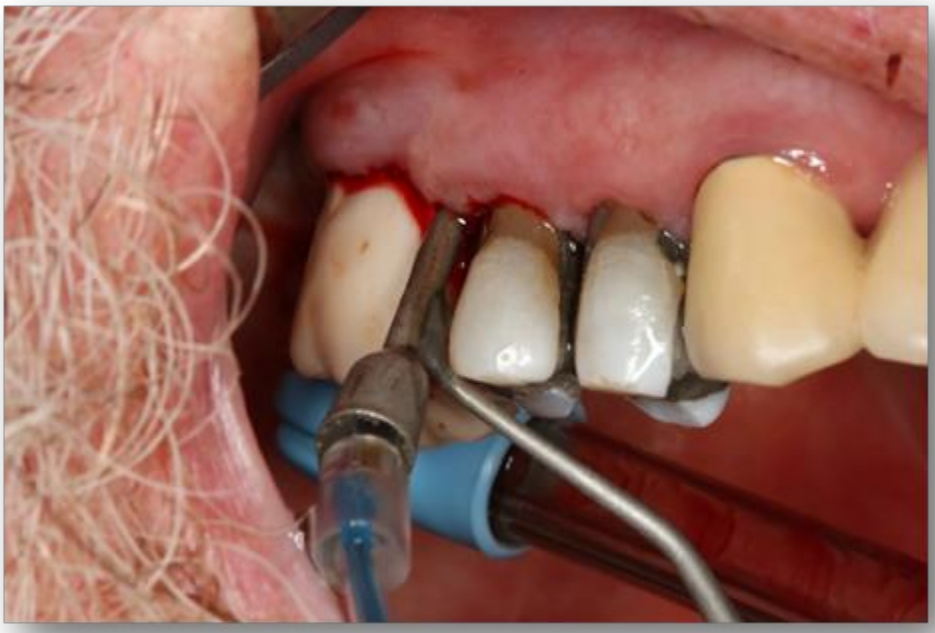
Following randomization, two 3 mL syringes; one with SIM and one with MCL, were joined via Luer-locking connector and exchanged 50 times to yield a homogeneous mixture of 2.2 mg SIM/0.15 mg MCL. The control group used MCL alone. The loaded syringe was attached to a 19-gauge blunt-end needle and the dose was 0.15 ml of SIM/MCL or 0.15 ml of MCL was deposited at the proximal experimental site, beginning at osseous level and extending coronally onto both proximal surfaces. Light pressure was applied with damp gauze to reapproximate the papilla and remove any excess medicament. Cyanoacrylate (Periacryl, GluStitch Inc., Delta, BC, Canada) was applied to the buccal and lingual papilla for stabilization and set with damp gauze. A registered hygienist (LA or MC) then completed a full-mouth periodontal maintenance cleaning excluding the surgical site.

Figure 4: Clinical Surgical Photos

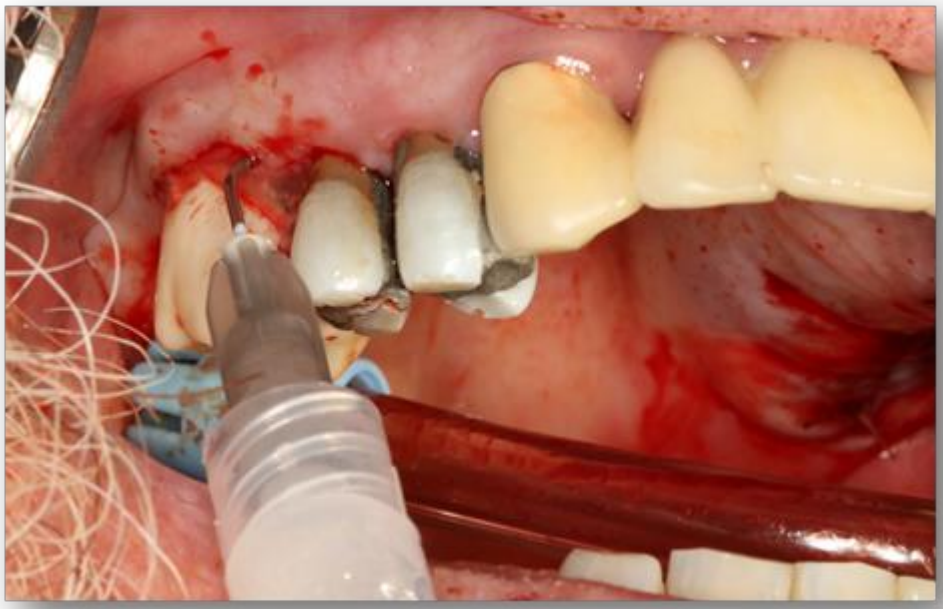
Baseline PD Measurement



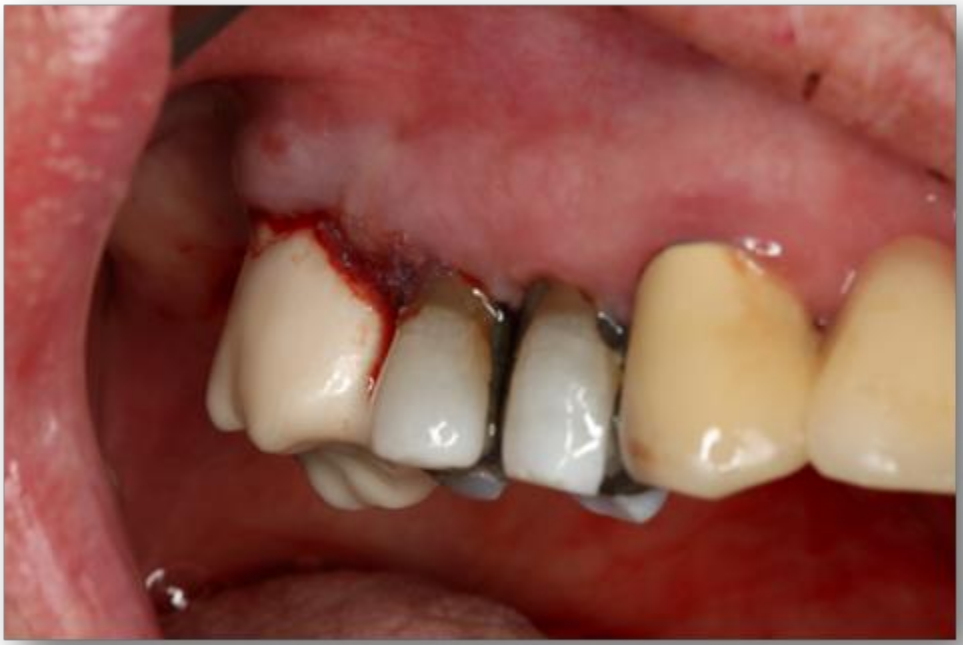
Papilla sparing incision with 12 blade



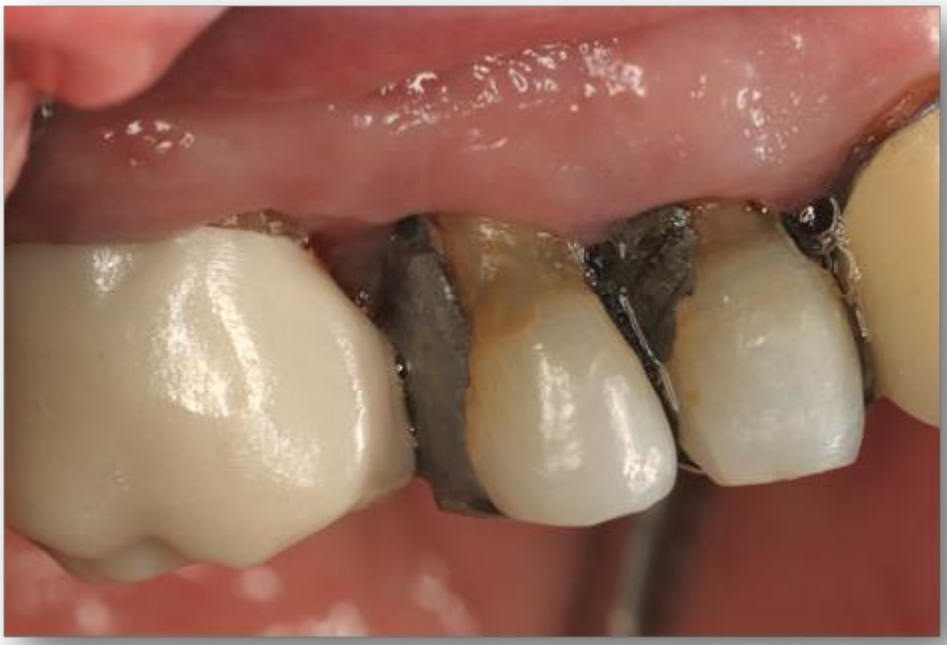
Endoscope to evaluate SRP



Simvastatin application



Papilla closure with cyanoacrylate



12-month post op

Postoperative Care

Following the procedure, patients were instructed to avoid brushing and interproximal cleaning of the experimental site for two weeks. Twice daily rinsing with Listerine (Johnson & Johnson, New Brunswick, NJ) for two weeks was advised. Patients returned for two- and six-week post-operative appointments. At both intervals patients were asked to report any experienced post-operative adverse events. At the two-week post-operative appointment, home care was reviewed with the recommendation to resume normal brushing of the experimental site. At the six-week post-operative appointment patients were instructed to begin daily interproximal brush use at the experimental site from both a buccal and lingual approach.

Patients also returned for routine periodontal maintenance with a registered dental hygienist at three-, six-, nine-, and 12-month intervals. The baseline clinical measurements were repeated at the 12-month visit (AK, RR, RH).

Statistical analysis

A sample size of 22 per group was needed to achieve at least 80% power to detect a difference of 1.0 mm in clinical attachment level between groups with a common estimated group standard deviations of 1.1 mm with a significance level of 0.05 using a two-sided two-sample t-test. This is based on mean data from relevant previous studies (Di Tullio et al., 2013; Killeen et al., 2016).

To qualify for enrollment in this study, patients must have had one interproximal site with a probing depth of 6-9 mm with history of BOP. For PD, CAL, and BOP measurements, the side of the treatment tooth with the deepest pocket at baseline was identified and only measurements from that side of experimental and adjacent teeth were analyzed; if buccal and lingual had equally deep pockets at baseline, then both measurements were averaged for each measure of interest.

Change in BOP was determined as follows: if the patient started without BOP and ended without BOP or showed a reduction (i.e. presence of BOP at baseline to absence of BOP at 12 months), that patient was considered to have a good outcome. If the patient began with BOP and showed no improvement or they developed BOP, that patient was considered to have a poor outcome. To verify reliability of IBH measurements, replicate measurements were made for ten patients, by the same assessor (LK); measurements were measured for treatment site and adjacent site at baseline and 12 months.

Associations between categorical variables were assessed using Chi-Square tests, or Fisher's exact tests. Medians and inter-quartile ranges (IQRs; the range of the middle 50% of the data (25^{th} percentile, 75^{th} percentile)) were calculated for each treatment condition, and Wilcoxon Rank Sum tests were used to examine differences in distributions of BOP between the two treatment conditions (i.e. SIM or MCL) for baseline BOP values. Means and standard deviations were calculated for age and PD and CAL measurements, and differences in baseline values between groups were assessed using t-tests.

Linear models were used to assess the association between the outcome change in measurement (twelve month – baseline) and group, while adjusting for the initial measurement and side of worst pocket. Model adjusted mean change estimates were calculated from these models. For the change in BOP outcome, logistic regression models were used, which included group and adjustment for worst side. Adjusted odds ratios are presented with 95% confidence intervals. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

CHAPTER 6: Results

Patient Characteristics

The 50 patients included in this study were eligible and consented to participate. The average age of patients was 66.3 (±10.4) for the test group and 65.5 (±7.2) for the control group with no significant difference in age between groups (p=0.76) (Table 1). There was no significant difference in gender enrollment between groups, but the test group had significantly more smokers (8 smokers) than the control group (1 smoker).

TABLE 1: Demographics between Groups (Data Utilized for Randomization)

	Simvastatin		Control		
	n=27	**%**	**n=23**	**%**	**p-Value**
Gender					0.64††
Female	10	50.0	10	50.0	
Male	17	56.7	13	43.3	
Smoking Status					0.03^
Non-smoker	19	46.3	22	53.7	
Smoker	8	88.9	1	11.1	
Mean Age	66.3 (±10.4)		65.5 (±7.2)		0.76†

† P-values from t-tests.

†† P-values from Chi-Square tests

^ P-value from Fisher exact test

Two patients did not complete the study in its entirety (4% dropout rate). Of the two patients that did not complete the study, one patient was diagnosed with lung cancer and the second patient failed to comply with appointment attendance. Neither reason for patient dropout was believed to be related to any dental treatment provided throughout the study.

Forty-eight patients participated in the study for 12 months. All participants were questioned as to what adverse symptoms were experienced at two-week, six-month, and 12-month intervals. Overall, very minor and few symptoms were experienced. Two weeks into the

study, nine patients reported sensitivity to temperature, six reported some pain, and one reported swelling. By twelve months, only one patient reported pain and all temperature sensitivity and swelling had resolved.

Clinical Outcomes

The mean baseline and 12-month final post-treatment results for respective changes in clinical outcomes between groups over time are reported in Tables 2-7. As outlined in Table 2, there were no significant differences between groups for baseline PD (p= 0.55) or CAL (p=0.80). Both the PR/RP+SIM/MCL and the PR/RP+MCL groups saw a reduction in PD (-2.34 mm ± 0.26, p<0.0001; -1.34 mm ± 0.28, p<0.0001) and gain in CAL (1.93 ± 0.31 mm, p<0.0001; 1.01 ± 0.32 mm, p<0.003) from baseline to 12 months at the treatment, with a significant difference between groups (PD: p= 0.007; CAL: p= 0.03).

TABLE 2: Clinical Outcomes at Treatment Sites (change on side of deepest pocket depth)

Variable	SIM/MCL Baseline Mean (mm ± SE)	MCL Baseline Mean (mm ± SE)	T- test P-value	Group	Model Adjusted Mean Change (12 months-Baseline) (mm ± SE)	P- Value for changes from baseline within group	P-value for difference in change between groups
PD	6.2 ± (0.7)	6.4 ± (0.8)	0.55	SIM	-2.34 ± (0.26)	<.0001	0.007*
				MCL	-1.34 ± (0.28)	<.0001	
CAL	7.0 ± (1.4)	6.9 ± (1.2)	0.80	SIM	-1.93 ± (0.31)	<.0001	0.03*
				MCL	-1.01 ± (0.32)	0.003	

*indicates significant difference

PD: The distance from the soft tissue (gingiva or alveolar mucosa) margin to the tip of the periodontal probe.
CAL: The distance from the cemento-enamel junction to the tip of a periodontal probe during periodontal diagnostic probing.

As displayed in Table 3, adjacent interproximal sites to the treatment sites experienced improvements in PD (SIM/MCL: -0.77 ± 0.24 mm, p=0.002; MCL: -0.71 ± 0.24 mm, p=0.05) although smaller than the treatment sites, with no significant difference between groups (PD: p=0.86). No significant changes were seen in CAL at adjacent sites (SIM/MCL: -0.35 ± 0.33 mm, p=0.30; MCL: -0.20 ± 0.34, p=0.56).

TABLE 3: Clinical Outcomes at Adjacent Sites

Variable	Group	Model Adjusted Mean Change (Final - Baseline) (mm ± SE)	P- Value for changes from baseline within group	P-value for difference in change between groups
PD	**SIM/MCL**	-0.77 ± (0.24)	0.002*	0.86
	MCL	-0.71 ± (0.24)	0.005*	
CAL	**SIM/MCL**	-0.35 ± (0.33)	0.30	0.74
	MCL	-0.20 ± (0.34)	0.56	

*indicates significant difference

As summarized in Table 4, the direct buccal and lingual sites of the experimental teeth had no significant reductions in PD for either group (midfacial: PR/RP + SIM/MCL -0.03, p=0.84; PR/RP + MCL -0.11, p= 0.46; midlingual: PR/RP + SIM/MCL -0.34, p=0.01; PR/RP + MCL -0.24, p=0.07). For CAL of midfacial experimental sites, no significant changes were seen in the test or control groups (midfacial: PR/RP + SIM/MCL 0.10, p=0.63; PR/RP + MCL -0.11, p= 0.59). For CAL

of midlingual experimental sites, a small improvement was seen in both groups (midlingual: PR/RP + SIM/MCL -0.42, p=0.02; PR/RP + MCL -0.35, p= 0.06).

Table 4: Clinical Outcomes at Direct Buccal and Lingual of Experimental Sites

Variable	Group	Model Adjusted Mean Change (Final - Baseline) (mm ± SE)	P- value for changes from baseline within group	P- value for difference in change between groups
PD Midfacial	**SIM/MCL**	-0.03 ± (0.15)	0.84	0.67
	MCL	-0.11 ± (0.15)	0.46	
PD Midlingual	**SIM/MCL**	-0.34 ± (0.13)	0.01*	0.56
	MCL	-0.24 ± (0.13)	0.07	
CAL Midfacial	**SIM/MCL**	0.10 ± (0.20)	0.63	0.43
	MCL	-0.11 ± (0.20)	0.59	
CAL Midlingual	**SIM/MCL**	-0.42 ± (0.18)	0.42	0.77
	MCL	-0.35 ± (0.18)	0.97	

*indicates significant difference

The test group had a statistically significant change in BOP of experimental teeth from baseline to 12-month post therapy (p=0.047). As shown in table 5, patients treated with PR/RP + SIM+MCL (test) had 4.17 (95% CI AOR: 1.02, 17.04) times the odds versus the control (PR/RP+MCL) group of having a good bleeding outcome. A good BOP outcome means that the site showed improvement or maintained no BOP.

Table 5: Change in Bleeding on Probing of Experimental Sites

Outcome	Group	Adjusted^ Odds Ratio	95% Confidence Interval		P-Value for difference in change between groups
Exp					0.047*
	SIM/MCL	4.17	1.02	17.04	
	MCL	1.00	Reference		
Adj					0.57
	SIM/MCL	1.53	0.35	6.72	
	MCL	1.00	Reference		

*indicates significant difference

IBH measurements, displayed in Table 6, show that small gains were seen in both the test and control groups from baseline to 12 months (PR/RP+SIM/MCL -0.17 ± 0.15, p=; PR/RP+MCL -0.42±0.16, p=0.01). Although the improvement of IBH measurements in the control group was statistically significant, it was less than half a millimeter, therefore not clinically significant.

Table 6: Radiographic Interproximal Bone Height Outcomes at Experimental Site

	Model Adjusted Mean Change (Final – Baseline) (mm ± SE)	P-value for change from baseline within group	P-value for difference in change between groups
SIM/MCL	-0.17 (± 0.15)	0.28	0.22
MCL	-0.42 (± 0.16)	0.01*	

Plaque index displayed in Table 7, was determined via calculating the mean of 6 sites from the treatment tooth and six sites from the adjacent tooth, for a total of 12 sites. Both the PR/RP+SIM/MCL and PR/RP+MCL groups saw an insignificant reduction in the experimental

teeth plaque index (PR/RP+SIM/MCL: -11.26 ± 6.37m p=0.08; PR/RP+MCL: -9.09 ± 6.57 mm, p=0.17), with no significant difference between groups (p=0.80).

Table 7: Plaque Index of Experimental Sites

	SIM/MCL	MCL	p-value
Baseline Plaque %	59.5	47.8	0.08
Standard Deviation	25.3	20.6	

	Model Adjusted Mean Change (Final - Baseline) (± SE)	P-value for change from baseline within group	P-value for difference in change between groups
SIM/MCL	-11.26 (±6.37)	0.08	0.80
MCL	-9.09 (±6.57)	0.17	

Chapter 7: Discussion

This double-blinded, randomized, controlled clinical trial compared clinical outcomes of two therapies, PR/RP + SIM/MCL (test) and PR/RP + SIM (control), with local application in a 6-9 mm pocket in periodontal maintenance patients over a 12 month period. To minimize any bias between participants conducting this trial, the experimental steps were conducted as follows: one of three masked examiners (AK, RR, RH) collected data, surgical treatment protocols were completed by one of two clinicians (LK, MB) with the aid of one of two dental hygienists (LA, MC), one of two dental hygienists completed all PMT for the 12 month period, randomization was completed after PR/RP was performed to minimize bias that could affect instrumentation thoroughness, and all patients were masked as to which treatment group they were assigned. There were no significant differences of baseline clinical measurements between test and control groups. There were no conflicts of interest or support by manufacturer(s) of simvastatin for this study.

Change in CAL was the primary outcome measured in this study, with change in PD, BOP, and IBH as secondary measurements. The current study demonstrated treatment of an inflamed, 6-9 mm periodontal pocket in a periodontal maintenance appointment with both groups showing significant improvement in CAL ($p<0.003$), PD ($p<0.001$), BOP (SIM/MCL only; $p=0.047$). IBH was stable in both groups. A significant difference was found when comparing gain of CAL from baseline to 12 months post-treatment in the test group when compared to the control ($p=0.03$). There was also greater improvement in PD ($p=0.007$) and BOP ($p=0.047$) at the experimental sites in the test group when compared to the control group at 12 months. To our knowledge, no other studies have compared PR/RP + SIM/MCL to PR/RP + MCL as a part of periodontal maintenance therapy.

The PR/RP + SIM/MCL showed a CAL gain of -1.93 mm ± (0.31) and the PR/RP + MCL group showed a gain of -1.01 mm ± (0.32) over 12 months. The current study also showed improvement in PD in both groups. The PR/RP + SIM/MCL showed a PD improvement of –2.34 mm ± 0.26, which was a significant improvement compared to the control group reduction of –1.34 ± 0.28 (p=0.007), leaving the mean post-treatment CAL around 5 mm and PD around 4 mm in the PR/RP + SIM/MCL group.

Direct comparison of the results of this study to other local SIM application studies is challenging due to it being the only study in the periodontal maintenance population. Previous studies have measured change in clinical parameters (PD, CAL, BOP, IBH) during scaling and root planing therapy, which is the first line treatment performed in those with generalized inflammation of periodontal tissues (Pradeep et al., 2010; Priyanka et al., 2017). Conversely, periodontal maintenance is performed on those that have already undergone root planing and whose overall periodontal inflammation has reached stability or been reduced. It is intuitive then that any adjunctive treatment administered at the time of initial therapy, where inflammation is uncontrolled, would result in a greater improvement of clinical parameters over adjunctive therapies added to residual pockets in periodontal maintenance patients, where the majority of inflammation has already been reduced by instrumentation and homecare. The improvements in PD and CAL seen in previous local application SIM studies were greater than those found in this study. Pradeep et al. (2010) reported a decrease in PD of 4.26 ± 1.59 mm in the test group compared to a PD reduction of 1.20 ± 1.24 mm in the control group. The same study found a CAL gain of 4.36 ± 1.92 mm in the treatment group that received SRP in 5-≥7 mm pockets plus 1.2mg local SIM application versus CAL gain in the placebo group of 1.63 ± 1.99 mm. Another study that assessed effectiveness of SIM local delivery as adjunct to SRP, reported a PD decrease of 3.78 ± 0.62 mm in the test group and 1.14 ± 0.04 mm in the control (Priyanka

et al., 2017).

The PD improvement at midlingual sites on experimental teeth was statistically significant, but the clinical ramifications are nominal and fall within measurement error of ± 1 mm (Osborn et al, 1992; Corraini et al., 2013). All of the changes seen in PD and CAL at adjacent, midfacial, and midlingual sites were small (<1 mm). With majority of these changes being positive, yet small, they may be attributed to the Hawthorne Effect, and the patients' improvement in homecare due to knowledge of participating in a clinical study.

Previous studies conducted have shown significant radiographic bone fill in the test group receiving local SIM application versus a placebo (Priyanka et al., 2017; Rath et al., 2012). However, an imperative variance to note when comparing other studies' radiographic bone height changes to the current study, is that vertical or intrabony defects were part of the other studies' inclusion criteria. This may play a factor in the amount of regeneration which occurred, as defects with a depth of >3 mm and a radiographic defect angle of 25 degrees were reported to be the most amenable to regenerative procedures (Tonetti et al., 1993). The current study did not include vertical defects, but instead utilized residual pockets with horizontal bone loss defects. The difference in defect morphology could likely be attributed to bone fill seen in other studies compared to lack of significant gain in IBH in the test group of the current study.

A significant reduction in BOP was seen in the current study in the test group when compared to the control, which can be attributed to the anti-inflammatory effects of SIM that have also been corroborated in previous research. In this study, sites treated with PR/RP+SIM had a 4.17 (95% CI AOR: 1.02, 17.04) times the odds than the control (PR/RP + MCL) group of showing and improvement or maintaining no BOP. A study by Pradeep et al. (2012) reported a statistically significant decrease in bleeding index at 6 months when comparing placebo group

(1.61 ± 0.43) to the test group (0.80 ± 0.18) that received local application of SIM combine with SRP (p=0.001).

There were several limitations in this study. A major limitation was conducting this trial during a covid-19 pandemic. Patients were late on receiving 9 month and 12 month PMT appointments. During a post-study audit, six patients were found to have been assigned to the wrong study group according to the randomization table. Both groups had the requested number of patients according to the sample size calculations despite the error in the six patients. Also, more smokers, a major risk factor of periodontitis, were assigned to the SIM/MCL group. This may have decreased the positive effects in the SIM/MCL group. Both treatment modalities in this study utilized the aid of the endoscope during instrumentation. The enhanced visualization provided by the endoscope could have potentially changed the overall effects that were seen in the two treatments due the superior root debridement that is allowed. In spite of these limitations, SIM/MCL overcame these factors to provide statistically sand clinically significant improvements while adding approximately 20 minutes to a PMT appointment.

The combination of SIM and MCL is not commercially available in the United States and, therefore, required aid of a compounding pharmacy for use in this trial. Due to MCL being a non-toxic, non-allergic, and non-irritating material, it is commonly used for a sustained-release vehicle for therapeutic drug applications (Final Report, 1986). Cellulose pulp is a component of plant cell walls and is where methylcellulose, which is commonly used for oral and topical pharmaceutical suspensions, is derived (Al-Kassas et al., 2009). Ideally, SIM and MCL would be readily available and FDA approved for local application in periodontal pockets to make its clinical use more practical for use in the periodontal practice.

Based on the results presented herein, this clinical trial is promising as to the potential benefit of locally applied simvastatin in residual, inflamed periodontal pockets in periodontal maintenance patients. The significant improvement in periodontal clinical parameters combined with the economical and ease of availability of simvastatin make its use in periodontal therapy practical in a clinical setting. Additional research is required to determine the best clinical application and benefits of local application of SIM in periodontal pockets. Longer term follow up to assess longevity of improved periodontal parameters, as well as the influence of SIM on inflammatory biomarkers and growth factors would all be beneficial in the investigation of the benefit of the clinical application of SIM in periodontal patients.

CHAPTER 8: Conclusion

Scaling and root planing with papilla reflection in inflamed, residual, deep periodontal pockets during PMT with or without the application of SIM, resulted in improvements in PD, CAL, and BOP with stability of IBH after 12 months of PMT. The addition of SIM significantly enhanced the clinical benefits of PR/RP treatment during a periodontal maintenance appointment with inflamed 6-9 mm probing depths. Further research should be conducted investigating the anti-inflammatory and anti-microbial effects of SIM, as well as long-term clinical effects.

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APPENDIX A: Raw Clinical Data

Raw Clinical Data – Patient Characteristics

Patient	Group	Age	Gender	Smoking
1	B	46	m	1
2	B	76	m	0
3	A	69	m	0
4	B	60	m	0
5	B	70	m	0
6	B	58	f	0
7	B	71	m	0
8	B	54	f	0
9	B	57	f	0
10	A	70	m	0
11	A	80	m	0
12	A	52	f	0
13	B	66	f	0
14	A	61	m	1
15	A	64	m	0
16	A	72	f	1
17	A	65	f	0
18	B	69	f	0
19	B	60	f	0
20	B	69	f	0
21	B	68	m	0
22	A	64	f	0
23	A	77	f	0
24	A	68	f	0
25	B	71	m	0
26	B	63	m	0
27	A	75	m	0
28	A	76	m	0
29	A	77	m	0
30	A	42	m	1
31	A	44	m	1
32	A	78	m	0
33	A	48	m	1
34	A	74	m	0
35	B	66	m	0
36	A	65	f	0
37	A	80	f	0

38	A	64	f	1
39	A	62	f	0
40	A	73	m	1
41	A	60	m	1
42	A	64	m	0
43	A	65	m	0
44	B	76	m	0
45	B	65	m	0
46	B	72	f	0
47	B	70	m	0
48	B	61	m	0
49	B	68	f	0
50	B	70	f	0
	A = SIM+MCL		m=male	0=non-smoker
	B= MCL		f=female	1=smoker

Raw Clinical Data – CAL Experimental Site Buccal and Lingual

Patient	Group	CAL experimental site facial	CAL experimental site lingual
1	B	6	7
2	B	7	7
3	A	4	5
4	B	6	7
5	B	6	8
6	B	6	6
7	B	4	6
8	B	6	9
9	B	6	6
10	A	6	6
11	A	4	6
12	A	7	7
13	B	5	6
14	A	8	8
15	A	6	6
16	A	4	6
17	A	5	8
18	B	3	7
19	B	9	10
20	B	8	8
21	B	7	6
22	A	7	9
23	A	6	8
24	A	5	5
25	B	7	6
26	B	7	9
27	A	8	6
28	A	4	7
29	A	6	6
30	A	7	6
31	A	6	6
32	A	6	11
33	A	5	8
34	A	6	7

35	B	4	5
36	A	9	9
37	A	6	8
38	A	5	7
39	A	5	6
40	A	4	6
41	A	6	6
42	A	4	6
43	A	6	5
44	B	4	7
45	B	6	4
46	B	4	7
47	B	4	7
48	B	6	6
49	B	4	6
50	B	6	6
	A = SIM+MCL	CAL recorded to nearest mm	
	B= MCL		

Raw Clinical Data – PD Experimental Site Buccal and Lingual

Patient	Group	PD experimental site buccal	PD experimental site lingual
1	B	5	6
2	B	7	7
3	A	5	6
4	B	6	5
5	B	4	7
6	B	6	6
7	B	4	6
8	B	6	9
9	B	6	6
10	A	4	6
11	A	3	6
12	A	6	6
13	B	5	6
14	A	7	6
15	A	6	6
16	A	3	6
17	A	3	6
18	B	3	6
19	B	7	7
20	B	6	6
21	B	6	6
22	A	6	7
23	A	5	8
24	A	5	5
25	B	7	6
26	B	6	7
27	A	6	5
28	A	3	6
29	A	6	6
30	A	6	5
31	A	6	6
32	A	3	8
33	A	3	7
34	A	6	7
35	B	4	5

36	A	5	6
37	A	5	6
38	A	4	7
39	A	5	6
40	A	4	6
41	A	6	6
42	A	4	6
43	A	6	5
44	B	4	6
45	B	6	4
46	B	3	7
47	B	3	6
48	B	5	6
49	B	4	6
50	B	6	6
	A = SIM+MCL	PD recorded to nearest mm	
	B= MCL		

Raw Clinical Data – PD Adjacent

Patient	Group	PD Adjacent Buccal	PD Adjacent Lingual
1	B	4	4
2	B	3	3
3	A	5	5
4	B	6	4
5	B	3	5
6	B	5	3
7	B	5	4
8	B	6	8
9	B	4	4
10	A	5	5
11	A	3	5
12	A	3	5
13	B	2	3
14	A	4	4
15	A	6	6
16	A	3	6
17	A	3	5
18	B	3	5
19	B	4	4
20	B	4	3
21	B	7	5
22	A	4	4
23	A	3	4
24	A	3	4
25	B	8	7
26	B	3	4
27	A	5	3
28	A	3	5
29	A	3	3
30	A	6	4
31	A	6	8
32	A	3	3
33	A	4	7
34	A	4	5
35	B	4	4

36	A	5	4
37	A	3	5
38	A	2	4
39	A	3	3
40	A	4	6
41	A	5	4
42	A	4	4
43	A	5	5
44	B	4	4
45	B	3	3
46	B	3	4
47	B	3	3
48	B	5	5
49	B	5	5
50	B	3	3
	A = SIM+MCL		
	B= MCL		

Raw Clinical Data - CAL Adjacent

Patient	Group	CAL Adj Buccal	CAL Adj Lingual
1	B	5	5
2	B	3	3
3	A	3	4
4	B	6	6
5	B	3	5
6	B	5	3
7	B	5	4
8	B	6	8
9	B	5	4
10	A	5	5
11	A	3	5
12	A	4	7
13	B	2	4
14	A	6	7
15	A	6	6
16	A	3	6
17	A	5	5
18	B	3	5
19	B	6	6
20	B	5	5
21	B	8	5
22	A	4	5
23	A	4	5
24	A	5	6
25	B	8	7
26	B	5	5
27	A	6	4
28	A	4	6
29	A	3	3
30	A	6	5
31	A	6	8
32	A	6	5
33	A	4	7
34	A	4	5
35	B	4	4

36	A	8	6
37	A	6	7
38	A	2	4
39	A	3	3
40	A	4	6
41	A	5	4
42	A	4	4
43	A	5	5
44	B	6	5
45	B	3	3
46	B	3	4
47	B	5	6
48	B	6	5
49	B	5	5
50	B	3	3
	A = SIM+MCL		
	B= MCL		

Raw Clinical Data - CAL – Experimental Tooth Direct Buccal and Lingual

Patient	Group	CAL Experimental Tooth Direct Buccal	CAL Experimental Tooth Direct Lingual
1	B	3	5
2	B	3	2
3	A	3	3
4	B	4	6
5	B	5	4
6	B	4	4
7	B	2	2
8	B	2	3
9	B	3	4
10	A	2	3
11	A	4	3
12	A	4	5
13	B	4	3
14	A	5	5
15	A	6	3
16	A	5	3
17	A	4	5
18	B	3	4
19	B	4	6
20	B	4	4
21	B	3	4
22	A	4	5
23	A	3	4
24	A	4	4
25	B	2	3
26	B	5	2
27	A	4	5
28	A	2	3
29	A	1	3
30	A	2	3
31	A	4	4
32	A	8	5
33	A	6	2
34	A	3	3
35	B	4	3

36	A	5	5
37	A	5	4
38	A	2	4
39	A	6	6
40	A	3	3
41	A	2	3
42	A	3	2
43	A	3	4
44	B	3	4
45	B	3	3
46	B	6	4
47	B	6	4
48	B	4	5
49	B	3	3
50	B	3	4
	A = SIM+MCL	CAL recorded to nearest mm	
	B= MCL		

Raw Clinical Data – BOP

Patient	Group	Baseline treatment site Buccal BOP	Baseline treatment site Lingual BOP	12-month treatment Buccal BOP	12-month treatment Lingual BOP
1	B	1	0	0	0
2	B	1	1	0	0
3	A	0	0	0	0
4	B	1	1	0	0
5	B	1	1	0	0
6	B	1	1	0	0
7	B	1	0	0	0
8	B	1	1	0	0
9	B	0	1	0	0
10	A	1	1	0	0
11	A	0	1	0	1
12	A	1	1	0	0
13	B	1	1	0	0
14	A	1	1	0	0
15	A	0	0	1	1
16	A	0	1	1	0
17	A	0	1	0	1
18	B	0	1	0	1
19	B	0	0	0	0
20	B	1	0	0	0
21	B	1	0	0	0
22	A	1	1	0	0
23	A	1	1	0	0
24	A	1	1	0	0
25	B	1	1	0	0
26	B	1	1	1	0
27	A	1	1	0	1
28	A	0	0	0	0
29	A	1	1	0	1
30	A	0	1	0	0
31	A	1	1	0	0
32	A	0	1	0	0
33	A	1	1	0	0
34	A	1	1	0	1
35	B	1	0	0	0
36	A	0	0	0	0
37	A	0	0	0	0

38	A	1	1	0	0
39	A	1	1	1	1
40	A	1	1	0	0
41	A	1	1	1	0
42	A	0	0	0	0
43	A	1	1	0	0
44	B	0	1	0	0
45	B	1	1	0	0
46	B	0	1	0	0
47	B	1	1	0	0
48	B	1	1	0	0
49	B	1	1	1	0
50	B	1	1	0	0
	A = SIM+MCL	1=present			
	B= MCL	0=absent			

Raw Clinical Data - IBH Experimental Site at Baseline and 12 month

Patient	Group	IBH EXP SITE BASELINE	IBH EXP SITE 12 MONTH
1	B	2.09	0.61
2	B	5.89	4.5
3	A	4.38	2.44
4	B	4.46	4.12
5	B	3.65	4.59
6	B	3.44	2.81
7	B	1.11	1.13
8	B	6.08	
9	B	4.77	4.52
10	A	4.26	3.57
11	A	3.8	3.79
12	A	5.65	4.64
13	B	3.74	3.25
14	A	5.62	6.46
15	A	2.62	3.32
16	A	3.6	3.44
17	A	3.49	4.69
18	B	6.94	5.6
19	B	5.46	5.58
20	B	3.1	3.31
21	B	4.44	3.42
22	A	5.79	4.47
23	A	5.74	4.44
24	A	4.45	4.45
25	B	5.62	4.36
26	B	2.27	2.58
27	A	3.4	4.22
28	A	3.94	3.86
29	A	3.1	2.91
30	A	2.7	2.53
31	A	4.71	4.45
32	A	5.48	5.82
33	A	6.46	6.15
34	A	3.92	3.66
35	B	4.88	5.01

36	A	7.77	6.42
37	A	4.64	5.32
38	A	7.42	
39	A	2.92	2.95
40	A	2.68	2.38
41	A	2.94	2.41
42	A	6.64	6.6
43	A	3.31	3.59
44	B	2.4	2.85
45	B	6.52	6.25
46	B	4.21	3.28
47	B	3.59	3.69
48	B	3.06	2.94
49	B	3.1	2.98
50	B	3.97	2.51
	A = SIM+MCL		
	B= MCL		

Raw Clinical Data - IBH Adjacent Site at Baseline and 12 month

Patient	Group	IBH ADJACENT SITE BASELINE	IBH ADJACENT SITE 12 MONTH
1	B	2.38	0.8
2	B	0.66	1.2
3	A	2.61	2.42
4	B	4.83	3.9
5	B		1.63
6	B	3.66	2.25
7	B	0.91	0.92
8	B	4.43	
9	B	3.15	3.93
10	A	4.07	3.94
11	A	2.4	2.37
12	A	5.88	6.48
13	B	2.79	3.35
14	A	3.25	4.56
15	A	3.56	3.59
16	A	3.09	4.79
17	A	6.08	7.23
18	B	2.85	4.52
19	B	3.99	4.47
20	B	3.44	4.1
21	B	3.42	3.34
22	A	1.22	2.31
23	A	1.11	2.05
24	A	3.27	3.05
25	B	5.62	4.62
26	B	1.61	1.46
27	A	1.31	2.88
28	A	3.97	3.74
29	A	3.07	3.13
30	A	2.79	2.61
31	A	4.62	4.75
32	A	5.05	5.77
33	A	3.05	3.1
34	A	3.32	3
35	B	4.83	5.19

36	A	5.25	5.25
37	A	3.68	5.56
38	A	3.05	
39	A	2.81	2.54
40	A	3.45	3.45
41	A	3.12	1.89
42	A	5.02	6.09
43	A	2.88	3.53
44	B	3.47	3.82
45	B	2.56	2.59
46	B	3.3	2.65
47	B	5.01	4.36
48	B	1.41	2.07
49	B	2.4	2.31
50	B	3.12	3.76
	A = SIM+MCL		
	B= MCL		

APPENDIX B: Patient Consent Form

University of Nebraska Medical Center

College of Dentistry

IRB PROTOCOL # 217-18-FB **Page 1 of 9**

ADULT CONSENT - CLINICAL BIOMEDICAL

Title of this Research Study
Effect of Locally-Applied Simvastatin on Clinical Attachment Level and Alveolar Bone in Periodontal Maintenance Patients

Invitation
You are invited to take part in this research study. You have a copy of the following, which is meant to help you decide whether or not to take part:

- Informed consent form
- "What Do I need to Know Before Being in a Research Study?"
- The Rights of Research Subjects

Why are you being asked to be in this research study?
You are being asked be in this study because you are 40-85 years old and have periodontal disease.If you are pregnant, nursing an infant, or plan to become pregnant during this study, you may not be in this study.

What is the reason for doing this research study?
The scientific purpose of this study is to determine the effect of simvastatin in patients with periodontal bone loss who are on a regular periodontal maintenance routine.

Simvastatin is an FDA-approved drug shown to enhance bone growth in patients with periodontitis.

What will be done during this research study?
If selected for this study, you will be randomized (similar to flipping a coin) to one of the two study groups. One study group will receive a dose of simvastatin-methylcellulose (FDA-approved) to a site with bone loss between two teeth. The other study group will receive a dose of methycellulose (placebo) to a site with bone loss between two teeth.

You will have a radiograph (x-ray) taken of the tooth with the bone loss. Measurements (in millimeters) of the space between the tooth and the gums will be taken of the study site. Small paper points will be used to soak up the fluid from the space between the tooth and the gums. You will then receive a small injection of local anesthesia, followed by lifting of the gum tissue in the area of the bone loss. The study drug (simvastatin in methylcellulose) or methylcellulose will be placed in between the tooth and the gums after the tooth has been cleaned. The gum tissue

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will be put back into place and secured with dental glue. The rest of the teeth will then be cleaned as typical of a periodontal maintenance appointment. You will be seen approximately two weeks later to make sure that the study site is healing well. You will continue on your typical periodontal maintenance schedule and will have a new radiograph and new sample of fluid taken of the study tooth in 12 months.

If you are female, we will have you take a urine pregnancy test to ensure you are not pregnant. Upon signing the consent form and a negative pregnancy test (for female participants), an x-ray will be taken, followed by measurements and sampling of fluid from study site, small injection of anesthesia, lifting of gum tissue, placement of the study drug or methylcellulose, closure of the study site with dental glue and cleaning of all remaining teeth in the mouth (periodontal maintenance therapy) and will take approximately 90-120 minutes.

At the two-week follow-up visit, the study site will be checked for proper healing and to answer any questions. A fluid and tissue sample will be taken from the healing site. This visit will take approximately 15-30 minutes.

At the six-week follow visit, the study site will be checked for proper healing and to answer any questions. This visit will take approximately 15-30 minutes.

The 3, 6, and 9-month periodontal maintenance visits will be simple, routine maintenance appointments that will include full-mouth probing of teeth, cleaning of all teeth, polishing and flossing. The appointments take approximately 45 minutes.

The 6-month periodontal maintenance appointment will include a fluid sample of the study tooth,full-mouth probing of all teeth followed by routine periodontal maintenance (cleaning, polishing, flossing) of all teeth. This appointment will take approximately 60 minutes..

The 12-month periodontal maintenance appointment will include a radiograph and fluid sample of the study tooth, full-mouth probing of all teeth followed by routine periodontal maintenance (cleaning, polishing, flossing) of all teeth. This appointment will take approximately 60 minutes.

Information recorded at each visit will include periodontal probing depth, recession and bleeding on probing.

Typical and standard procedures for periodontal maintenance include the taking of radiographs, full-mouth periodontal probing (measuring the space between the teeth

and the gums), scaling and root planing (removing debris from the tooth surface both above and below the gumline), polishing the teeth and review of home care practices (brushing, flossing). These procedures are part of the standard periodontal maintenance and are not related to the research being conducted.

The anesthesia, lifting of the gums, placement of either the study drug or the placebo, use of dental glue and paper point fluid sampling are procedures for experimental purposes only and are part of the research being conducted. These procedures are not typical of periodontal maintenance appointments. However, anesthesia, lifting of the gums and use of dental glue are standard of care and very common in surgical periodontal procedures.

The information recorded from the medical record for the purposes of this research include the measurements of the space between the tooth and the gum and whether the site lightly bled upon being measured.

What are the possible risks of being in this research study?
No risks are associated with the paper strip. Simvastatin is FDA-approved and will be given at a significantly lower dose than when typically used systemically. Lifting the gum tissue is within the standard of care for 6-9 mm inflamed periodontal pockets (inclusion criteria). The potential risks associated with the simvastatin are no different than those associated with routine surgical periodontal therapy: 1) local infection, 2) mild-moderate discomfort, 3) bleeding.

The risk of loss of confidentiality is low due to the few number of investigators as well as the privacy of the office where consent will be obtained and the clinic where the data will be collected. The locked filing cabinet and locked room in the Cruzan Center where the data will be kept will also contribute to a low risk of loss of confidentiality.

It is possible that the medicines used in this study could injure a fetus if you, or your partner becomes pregnant while taking them. You have already been told what is known about this possibility, and you are encouraged to ask further questions.

You may want to discuss this with others before you agree to take part in this study. If you wish, we will arrange for a doctor, nurse, or counselor who is not part of this study to discuss the potential risks and benefits with you and anyone else you want to have present.

Because of the potential risks, you or your partner, must not become pregnant while you are participating in the study. Women must have a negative pregnancy test

before entering the study and receiving the study-related treatment.

If you are sexually active and can get pregnant, or can get your partner pregnant, you must use ONE appropriate method of birth control every time you have sex, or you must not have sex.

Because of the nature of this research, methods of natural family planning are not, by themselves, sufficiently reliable to avoid pregnancy.

You can get additional information about methods to avoid pregnancy by calling the UNMC Research Subject Advocate's Office at (402) 559-6941.

You will need to continue to avoid pregnancy for 6 months after finishing the research.

By signing this and being in the study, you are agreeing to not get pregnant while you are on the study and for 6 months after completion of the study. Should you become pregnant while on this study, you should immediately notify the study personnel. The investigator will assist you in finding appropriate medical care. The investigator also may ask to be allowed to continue getting information about your pregnancy. You can refuse to provide this information.

It is possible that other rare side effects could occur which are not described in this consent form. It is also possible that you could have a side effect that has not occurred before.

What are the possible benefits to you?
Possible benefits of being in this study could include bone regrowth or a less inflammation in the area of the bone loss.
You may not get any benefit from being in this research study.

What are the possible benefits to other people?
This study could possibly benefit society by examining another way to treat bone loss, which could ultimately lead to patients retaining their own teeth for a longer time.

What are the alternatives to being in this research study?
The alternative to being in this study would be periodontal surgery with or without chemotherapeutics.

What will being in this research study cost you?
You will be responsible for all fees related to your regular care and visits. You will not be charged for any visits or procedures that are for research purposes only.

Will you be paid for being in this research study?
You will be paid the equivalent of the standard allowable rate of $20/hour. The compensation will be in the form of either a Target or Visa gift card.

Who is paying for this research?
This research is being paid for by the Department of Surgical Specialties, Section of Periodontics at the University of Nebraska Medical Center College of Dentistry.

This research is being paid for by grant funds from the Windsweep Farm Fund.

What should you do if you are injured or have a medical problem during this research study?
Your welfare is the main concern of every member of the research team. If you are injured or have a medical problem as a direct result of being in this study, you should immediately contact one of the people listed at the end of this consent form. Emergency medical treatment for this injury or problem will be available at the Nebraska Medical Center. If there is not sufficient time, you should seek care from a local health care provider.

The Institution has no plans to pay for any required treatment or provide other compensation. If you have insurance, your insurance company may or may not pay the costs of medical treatment. If you do not have insurance, or if your insurance company refuses to pay, you will be expected to pay for the medical treatment.

Agreeing to this does not mean you have given up any of your legal rights.

How will information about you be protected?
You have rights regarding the protection and privacy of your medical information collected before and during this research. This medical information is called "protected health information" (PHI). PHI used in this study may include your medical record number, address, birth date, medical history, the results of physical exams, blood tests, x-rays as well as the results of other diagnostic medical or research procedures. Only the minimum amount of PHI will be collected for this research. Your research and medical records will be maintained in a secure manner.

Who will have access to information about you?

By signing this consent form, you are allowing the research team to have access to your PHI. The research team includes the investigators listed on this consent form and other personnel involved in this specific study at the Institution.

Your PHI will be used only for the purpose(s) described in the section "What is the reason for doing this research study"?

You are also allowing the research team to share your PHI, as necessary, with other people or groups listed below:

- The UNMC Institutional Review Board (IRB)
- Institutional officials designated by the UNMC IRB
- Federal law requires that your information may be shared with these groups:
    - The HHS Office of Human Research Protections (OHRP)
    - The Food and Drug Administration (FDA)

You are authorizing us to use and disclose your PHI for as long as the research study is being conducted.

You may cancel your authorization for further collection of PHI for use in this research at any time by contacting the principal investigator in writing. However, the PHI which is included in the research data obtained to date may still be used. If you cancel this authorization, you will no longer be able to participate in this research.

How will results of the research be made available to you during and after the study is finished?

Information obtained in the course of the research that will not be shared with you is in to which group (study drug versus methylcellulose) you have been randomized. By signing this authorization, you are temporarily giving up your right to see this research-related information while the research is going on. You will be able to see this information if you wish after the research is completed.

In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address: [*Dr. Amy C. Killeen, UNMC College of Dentistry, 40th and Holdrege, Lincoln NE]*

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What will happen if you decide not to be in this research study?
You can decide not to be in this research study. Deciding not to be in this research will not affect your dental care or your relationship with your dental providers or the College of Dentistry. Your dentist or hygienist will still take care of you and you will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?
You can stop participating in this research (withdraw) at any time by contacting the Principal Investigator or any of the research staff. Deciding to withdraw will otherwise not affect your care or your relationship with the investigator or this institution.

You could be withdrawn from the study if you show any signs of allergy or hypersensitivity, becomre pregnant, or demonstrate rapidly progressing periodontitis in the experimental quadrant requiring periodontal surgery or tooth extractions or if you are non-compliant with the required visits. Any research data obtained to date may still be used in the research.

Will you be given any important information during the study?
You will be informed promptly if the research team gets any new information during this research study that may affect whether you would want to continue being in the study.

What should you do if you have any questions about the study?
You have been given a copy of "*What Do I Need to Know Before Being in a Research Study?*" If you have any questions at any time about this study, you should contact the Principal Investigator or any of the study personnel listed on this consent form or any other documents that you have been given.

What are your rights as a research participant?
You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights, or want to discuss problems, concerns, obtain information or offer input, or make a complaint about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
  - Telephone: (402) 559-6463.
  - Email: IRBORA@unmc.edu
  - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
  - Telephone: (402) 559-6941
  - Email: unmcrsa@unmc.edu

Documentation of informed consent
You are freely making a decision whether to be in this research study. Signing this form means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects
- You have had your questions answered.
- You have decided to be in the research study.
- If you have any questions during the study, you have been directed to talk to one of the investigators listed below on this consent form.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject _____
Date _____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate.

Signature of Person obtaining consent _____
Date _____

Authorized Study Personnel
Principal
* Killeen, Amy
phone: 402-472-1441
alt #: 402-472-7848

degree: DDS

Secondary

* Hattervig, Robin
phone: 402-472-1368
alt #: 402-472-1368
degree: DDS

* Reinhardt, Richard (Rick)
phone: 402-472-1287
alt #: 402-472-1287
degree: DDS