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The Effect of Simvastatin on Periodontonal Inflammation in Periodontal Maintenance Patients

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THE EFFECT OF SIMVASTATIN ON PERIODONTONAL INFLAMMATION IN PERIODONTAL MAINTENANCE PATIENTS

by

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Growing up I always knew I wanted to be a dentist. I fell in love with dentistry while working with my uncle who is an oral surgeon. As I reflect back on college, dental school and now residency, I have learned so much and have so much to be thankful for.

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THE EFFECT OF SIMVASTATIN ON PERIODONTAL REGENERATIVE MATERIAL ON INFLAMMATION IN PERIODONTAL MAINTENANCE PATIENTS

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The purpose of this analysis of inflammation markers from a double blinded, randomized, controlled clinical trial was to determine if local application of simvastatin, combined with minimally invasive papilla reflection/root preparation (PR/RP) is effective in reducing markers of inflammation: 1) bleeding upon probing (BOP), 2) tissue inflammatory / anti-inflammatory gene activation (rt-PCR) and 3) corresponding gingival crevicular fluid (GCF) protein production in non-resolving 6-9 mm periodontal pockets in patients on periodontal maintenance therapy (PMT). Fifty periodontal maintenance patients diagnosed with advanced chronic adult periodontitis presenting with a 6-9 mm interproximal PD were included in study. Experimental (PR/RP+SIM; n=27) and control (PR/RP+S, n=23) therapies were randomly allocated. Inflammation was assessed by bleeding upon probing (BOP) at baseline and 12-months and gingival crevicular fluid (GCF) samples at baseline, 2-weeks, and 12-months. To assess gene activation (RT-PCR), an approximately 2x2x2 mm piece of gingival connective tissue was harvested and assessed at baseline and 2-weeks postoperative therapy. Scaling and root planing with papilla reflection in inflamed, persistent, deep periodontal pockets during PMT with the addition of SIM, resulted in clinical improvements in BOP after 12-months. GCF Il-6 and VEGF were significantly elevated at 2-weeks wound healing, and an increase in 2-week GCF IL-10 was significantly correlated with improved CAL ($r=-0.32$, $p=0.03$), and rt-PCR raw values were numerically higher in SIM group at 2-weeks for IL-6, RANKL and IGF-1.

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BOP	bleeding on probing
CAL	clinical attachment level
PD	probing depth
REC	gingival recession
SIM	simvastatin
MCL	methylcellulose
SRP	scaling and root planing
GCF	gingival crevicular fluid
rt-PCR	real-time polymerase chain reaction
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
PR	papilla reflection
RP	root preparation
IBL	interproximal bone loss
PMT	periodontal maintenance therapy
FGF-2	fibroblast growth factor
IL-1β	interleukin-1 beta
IL-6	interleukin-6
IL-10	interleukin-10
IL-17A	interleukin-17a
IGF-1	insulin-like growth factor-1
TNF-α	tumor necrosis factor alpha
RANKL	receptor activator of nuclear factor kappa-B ligand
VEGF-A	vascular endothelial growth factor A

CHAPTER 1: INTRODUCTION

Periodontitis is a chronic inflammatory disease of the periodontal tissues resulting in clinical attachment loss, alveolar bone loss, and periodontal pocketing. It is estimated that 42% of adults over the age of thirty in the United States are affected (Eke et al. 2018). Diagnosis of periodontitis is made based on the following parameters: presence or absence of inflammation, severity of bone loss and attachment loss, periodontal pocketing, extent and pattern of teeth involved, medical and dental histories, pain, ulceration, and amount of plaque and calculus present (AAP Position Paper, 2003).

Periodontal destruction is driven by an inflammatory response to bacterial biofilm. Bleeding on probing (BOP), suppuration, increased volume and inflammation markers in gingival tissue and adjacent crevicular fluid (GCF) are all indications of inflammation.

There are various treatment options for periodontitis, which often include non-surgical and/or surgical therapies. Non-surgical therapy includes the removal of bacterial biofilms, calculus and cementum through scaling and root planing (SRP) and has been shown to improve clinical parameters as well as reduction of inflammation (Kaldahl et al., 1996a, Becker et al., 2001). In areas that do not respond to traditional non-surgical therapy, a surgical approach may be more beneficial. Surgical therapy may include the following: open flap and debridement, guided bone and tissue regeneration, apical positioned flaps, and or osseous resective surgery.

When a patient is considered periodontal stable, they are placed into a periodontal maintenance therapy (PMT) program consisting of dental visits every 3-4 months. The purpose of PMT is to maintain patient's oral health and to monitor their periodontal disease. Periodontal maintenance therapy is critical to the long-term success in treating periodontitis (Becker et al., 1984a, Becker et al., 1984b).

When a patient becomes unstable (recurrent inflamed pockets), the use of further surgery at a later appointment may be indicated. Methods which allow reduction of deeper pockets (e.g. 6-9mm) with bleeding on probing (BOP) within a PMT visit should increase patient acceptance and improve long-term outcomes.

The hypothesis of this study was that minimal papilla reflection/root preparation and simvastatin (SIM) + methylcellulose (MCL) would better reduce markers of inflammation compared to the same procedure with MCL alone in residual deep pockets during PMT. The study was registered with ClinicalTrials.gov (NCT03452891) and approved by the University of Nebraska Medical Center Institutional Review Board (#217-18-FB).

CHAPTER 2: LITERATURE REVIEW: SIMVASTATIN

The goal of periodontal therapy is to reduce inflammation and restore the periodontal tissues that have been previously lost or diminished. Clinical criteria, such as bone fill in osseous defects, gain in clinical attachment level (CAL) and reduction in bleeding upon probing (BOP) are the best ways to determine successful periodontal therapy (Consensus Report, 1998).

Simvastatin (SIM) belongs to a class of lipid-lowering medications that are specific competitive inhibitors of 3-hydroxy-2-methyl-glutaryl coenzyme A (HMG-CoA) reductase (Todd et al., 1990). Simvastatin is widely used in the medical field to lower cholesterol and also provide an effective approach for treating hyperlipidemia and arteriosclerosis (Hunninghake, 1998). Statins have been shown to modulate bone formation by increasing expression of bone morphogenetic protein-2 (BMP-2), decreased inflammation, and promote angiogenesis (Mundy et al., 1999).

Within the last several decades, SIM has been shown to enhance periodontal clinical attachment and bone levels and reduce inflammation in rats and humans when applied locally (Morris et al., 2008, Pradeep et al., 2010, Killeen et al., 2012, Krell et al. 2021). In 2002, a single high dose local application of simvastatin gel was shown to stimulate bone growth in rats (Thylin et al., 2002) with some surrounding soft tissue damage, and Stein et al. (2005) showed that a lower SIM dose reduced tissue damage without sacrificing bone-growth potential. Rutledge et al. (2011) investigated SIM application that mimicked a periodontal defect. Local application of SIM was placed in dehiscence defects adjacent to the roots of teeth in dogs and resulted in bone induction. Other studies found that a SIM prodrug, with or without anti-resorptive agents, promoted anti-inflammatory and bone anabolic effects (Killeen et al., 2012; Price et al., 2013; Bradley., 2016).

There are a limited number of human clinical studies that have reported on the effects of SIM in the treatment of periodontitis. Pradeep et al. (2010) showed that when SIM is delivered locally using a MCL carrier in patients with chronic periodontitis, there was a greater reduction in gingival bleeding, probing depths, and gain in clinical attachment level with significant intrabony bone fill that was lost due to periodontal destruction at sites treated with SRP plus SIM. A similarly designed study by the same group examining SIM's effect in treating molar Class II furcation defects in 72 patients resulted in a similar reduction of gingival inflammation and greater mean percentage bone fill in SIM-treated subjects (Pradeep et al., 2012). The authors concluded that locally delivered SIM provides a comfortable and flexible method to improve clinical parameters and also enhance bone formation.

A more recent clinical study that was done during the initial therapy phase (SRP), showed that the use of SIM in a local periodontal defect showed an increase in the clinical attachment level and increased intrabony defect fill when compared to traditional scaling and root planing alone (Priyanka et al., 2017). Two recent systematic reviews (Muniz et al., 2018, Amrosio et al., 2018) of SIM as adjunct therapy in localized periodontal defects showed that defects treated with SIM had improved probing depths, clinical attachment levels and bone fill. Results from the above studies, suggest that local delivery of simvastatin may be beneficial in the treatment of chronic periodontitis.

Achieving high intrasulcular drug concentration, avoiding the drug's systemic side effects, and better patient compliance are all benefits of using a subgingival drug delivery system (Goodson et al., 1985,). In order for SIM to be delivered locally, a carrier must be used. Various vehicles such as methylcellulose (MCL) have been studied and used in the literature for controlled-drug release. Methylcellulose (MCL) is commonly used in a variety of oral and topical

pharmaceutical formulations (Al-Kassas et al., 2009). It is also used widely in cosmetic and food products. Methylcellulose (MCL) is a non-toxic, non-allergic, and non-irritating material that is used as a sustained-released vehicle for various therapeutic drugs (Final Report, 1986).

In summary, the use of SIM+MCL in a flapless local application has been shown to increase radiographic bone height in intrabony defects, although the published radiographic evidence is sparse. Further, SIM/MCL as an adjunct to SRP has been shown to improve CAL and BOP, particularly as part of initial therapy (Pradeep et al., 2010). Unfortunately, SIM+MCL is not commercially available in the United States, and its use during PMT is lacking. Finally, analysis of markers of inflammation in surrounding tissues and fluids and the use of locally delivered SIM are understudied.

CHAPTER 3: LITERATURE REVIEW: ANALYTES

The host inflammatory response of periodontal disease involves both innate and adaptive immunity (Hajishengallis and Kristoff, 2017). Interactions between the innate and adaptive immune response involve inflammatory / anti-inflammatory cytokine and growth factors. Cytokines are cell-signaling molecules produced by immune cells that activate effector mechanisms. Cytokines play an important role in cell-to-cell communications in immune responses, can stimulate cells to move towards the site of inflammation, and they can have pro-inflammatory and anti-inflammatory effects. An increase in pro-inflammatory cytokine production may lead to more destruction within the periodontium. Also, growth factors may have anabolic effects on periodontal soft tissues and bone.

In this study the following growth factors and cytokines were analyzed: fibroblast growth factor (FGF-2), insulin-like growth factor 1 (IGF-1), interleukin (IL)-1β, IL-6, IL-10, IL-17A, tumor necrosis factor (TNF)-α, receptor activator of nuclear factor kappa-B ligand (RANKL), and vascular endothelial growth factor A (VEGF-A).

Fibroblast growth factor (FGF-2)

Fibroblast growth factor (FGF-2) is a naturally occurring protein that regulates cell growth and development (Lind, M. 1996). This growth factor exhibits potent angiogenic activity and mitogenic ability on mesenchymal cells within the periodontal ligament and has been shown to be effective in regenerating periodontal tissue in various human and animal models (Takayama et al, 2001, Murakami et al., Kitamura et al., 2011).

Insulin-like growth factor (IGF-1)

IGF-1 is a growth factor hormone that is similar to insulin which plays an important role in childhood growth and has an anabolic effect in adults (Laron, Z. 2001). IGF-1 has been shown to be a key player in periodontitis due to its anabolic factors that are responsible for limiting destruction caused by periodontitis (Okada et al., 1998). This growth factor has been shown to regenerate bone in human periodontal defects (Devi et al., 2016), and can also stimulate regeneration of the periodontal ligament (Halper, 2014). Han et al. showed that IGF-1 is capable of stimulating not only periodontal ligament fibers, but also stimulating cell proliferation, and local osteoblast precursor proliferation, differentiation, and mineralization of new bone (Han et al., 2003). When simvastatin is delivered locally in a ligature-induced periodontitis model, an activation of IGF-1 was seen (Liu et al., 2019). Therefore, SIM may play a crucial role in reversing periodontitis.

Interleukin 1β (IL1-β)

Interleukin 1β (IL1 β) is pro-inflammatory cytokine that is released in response to bacteria and their by-product (Cochran 2008). This interleukin is predominately produced by macrophages and monocytes. IL-1β plays an important role in inflammation, immune regulation, and bone resorption in periodontitis (Cheng et al., 2020). Various research has shown that increased levels of IL-1β are detected in the saliva and gingival crevicular fluid (GCF) of patients with periodontitis when compared with non-periodontal patients (Kinney et al., 2014, Rangbulla et al., 2017). A study done in 2013 by Sánchez et al. showed that patients with deeper probing depths and severe bleeding on probing (BOP) had increased levels of IL-1β. Patients with chronic periodontitis had higher IL-1β concentrations during episodes of periodontal inflammation (Lamster et al., 1992). In regards to bone resorption, IL-1β promotes osteoclast formation and is a potent inducer of bone demineralization (Dewhirst et al., 1985).

Periodontal sites with alveolar bone loss were associated with increased levels of IL-1β (Lee et al., 1995, Rogers et al., 2002). It is important to keep periodontal inflammation at a minimum, and after routine periodontal maintenance therapy (PMT), IL-1β levels decrease significantly (Hou et al., 1995).

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is an important pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection (Tanaka et al., 2014). This cytokine is well documented as a key player in periodontal disease (Irwin et al., 1998). Excess amounts of IL-6 are produced locally in patients with chronic periodontitis (Bozkurt et al., 2000). IL-6 is produced by periodontal ligament cells and is regulated by IL-1β and has been revealed a potentially important mechanism for controlling alveolar bone resorption (Shimizu et al., 1992). Salivary levels of IL-6 were significantly higher in patients with calculus associated chronic periodontitis when compared to healthy subjects. IL-6 increases as the disease progresses from mild to moderate and severe forms of periodontitis. (Batool et al., 2018).

Interleukin-10 (IL-10)

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine that suppresses both immunoproliferative and inflammatory responses (Zhang et al., 2014). IL-10 is produced by many different cell types including B cells, mast cells, eosinophils, macrophages, and dendritic cells, and a large number of subsets of T cells (O'Garra et al., 2008). As an anti-inflammatory cytokine, IL-10 plays an important role in periodontal disease. It has been shown that IL-10 inhibits osteoclastic bone resorption and regulates osteoblastic bone formation (Zhang et al., 2014). A study done by Glover et al. (2016) showed that when SIM is delivered subgingivally in humans, IL-10 is stimulated in the GCF around periodontal pockets and periodontal attachment

improves. A study done in mice by Sasaki, H. et al. (2004), showed that mice are highly susceptible to bone loss induced by the microorganism *Prophyromonas gingivalis*. Therefore, it was suggested that when SIM was delivered subgingivally, an increased expression of IL-10 was seen and in turn facilitated alveolar bone regeneration and limited the progression of periodontitis.

Interleukin-17A (IL-17A)

IL-17 is a pro-inflammatory cytokine which plays a vital role in a variety of processes, including host defense, tissue repair, and the pathogenesis of inflammatory diseases such as periodontitis. IL-17 induces multiple pro-inflammatory mediators, including chemokines, cytokines, and metalloproteinases from epithelial and fibroblast cells (Kolls et al., 2004). This cytokine is produced by activated CD4+ T cells (Yao et al., 1995) and is also a potent pro-osteoclast activator that has been linked to the pathogenesis of periodontitis (Zenobia et al., 2015). Takahaski et al. (2005), showed that IL-17 is locally produced by T cells in periodontal lesions and that IL-17 may exacerbate inflammatory reactions both directly and indirectly via inflammatory mediators from gingival fibroblasts within periodontal tissues.

Tumor necrosis factor (TNF- α)

TNF-α is a potent osteoclastogenic cytokine that is produced during an inflammatory response (Lam et al., 2000). As a pro-inflammatory cytokine, TNF- α plays an important role in the progression of periodontitis. TNF- α promotes bone loss that is seen in periodontitis by activating the expression of the RANK-L pathway (Hienz et al., 2015). Assuma et al. (1998) investigated the functional role of IL-1 and TNF in bone loss caused by experimental periodontitis by blocking IL-1 and TNF. By inhibiting IL-1 and TNF activity, the number of osteoclasts formed was reduced by 67%, the area of alveolar bone loss was inhibited by 60%,

and alveolar bone height lose was inhibited by 90%. Overproduction of IL-1 and TNF by the innate host defense is a major contributor to periodontal bone destruction. In regard to SIM and TNF- α, Liu et al. observed in rats, SIM treatment significantly repressed activity of TNF- α. Simvastatin exerts both anti-inflammatory and anti-osteoclastogenic effects by antagonizing expression of MMP-9 and TNF- α (Liu et al., 2019).

Receptor activator of nuclear factor kappa-B ligand (RANK-L)

Receptor activator of NF-κB ligand (RANK-L), a member of the tumor necrosis factor (TNF) ligand family, is responsible for stimulation of osteoclast differentiation and bone resorption (Lacey et al., 1998). RANK-L is produced as a membrane-bound or secreted ligand by osteoblasts, fibroblasts, and activated T- and B-cells. When RANK-L binds to its cognate RANK receptor on the surface of pre-osteoclasts, it drives differentiation into mature osteoclasts, thus in turn activating bone resorption. However, the action of RANK-L can be blocked by its soluble decoy receptor osteoprotegerin (OPG). By binding to RANK-L, OPG prevents its further interaction with RANK, and subsequently all the downstream molecular events that lead to osteoclast differentiation and bone resorption as seen in periodontitis (Belibaskis and Bostanci, 2012). Increased RANK-L or decreased OPG local expression can cause bone resorption, whereas decreased RANK-L or increased OPG local expression could result in bone formation. Therefore, the interaction between RANK-L and OPG plays an important role in the destruction of periodontitis. The importance of the interaction between RANK-L and OPG has been investigated in various studies. A study done in 2006 by Kawai et al. showed that TNF- α and IL-1 are present in the inflamed gingiva, and are able to induce RANK-L expression by osteoblast cells. Local expression of RANK-L was significantly higher in patients with periodontally-involved tissues when compared to healthy tissues.

Vascular endothelial growth factor A (VEGF-A)

Vascular endothelial growth factor A (VEGF-A) is a multifunctional angiogenic cytokine that plays an important role in inflammation and wound healing. VEGF is detectable in periodontal tissues within endothelial cells, plasma cells, and macrophages, and in junctional, sulcular, and gingival epithelium. There are several studies within periodontal literature that show the important role VEGF-A may play in periodontitis. Booth et al. (1998) examined patients with chronic periodontitis and the relationship of VEGF within GCF samples. The volume of GCF and total amount of VEGF were greater in diseased sites compared to clinically healthy sites. Johnson et al. (1999) showed that VEGF and IL-6 concentrations were significantly lower within healthy gingiva than within diseased sites, and the number of blood vessel profiles and mean IL-6 concentrations were highest in diseased sites. Therefore, Johnson et al. (1999) concluded that VEGF may play an important role in the initiation and progression of gingivitis to periodontitis, possibly by promoting expansion of the vascular network coincident to progression of the inflammation (Johnson et al., 1999). A study done by Prapulla et al. (2007), concluded that VEGF concentration in GCF increases proportionally with the progression of periodontitis.

Overall, these nine analytes have all been found to play an important role in pathogenesis of periodontitis, making these analytes an appropriate choice for GCF and tissue analysis.

CHAPTER 4: RESEARCH HYPOTHESIS AND SPECIFIC AIMS

The central research hypothesis is that minimal papilla reflection/root preparation and simvastatin (SIM) + methylcellulose (MCL) would better reduce markers of inflammation, and increase growth factors in the sulcus (BOP, GCF) and tissues adjacent to residual deep pockets (rt-PCR) during PMT.

Specific Aims

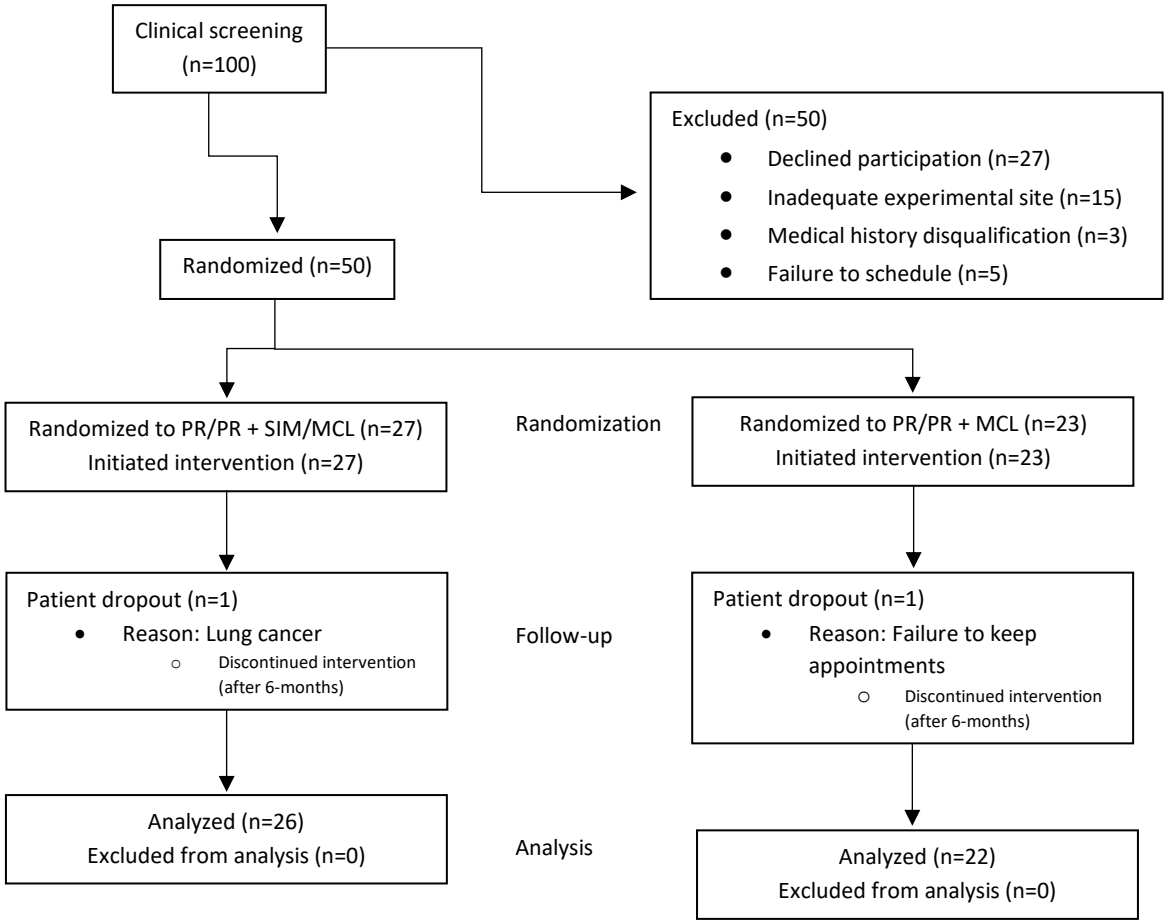
1. To determine whether SIM/MCL reduces the amount of inflammatory markers, increase anti-inflammatory markers, and growth factors in the GCF and gingival tissues of periodontal patients relative to a control (MCL), in inflamed 6-9 mm pockets during PMT.
2. To determine whether SIM/MCL reduces the frequency of BOP versus a control (MCL) in inflamed 6-9 mm pockets during PMT.
3. To determine if inflammatory markers during early wound healing are altered or correlated to later clinical outcomes.
4. To determine if inflammatory markers during early wound healing are correlated between gene activation in tissue (rt-PCR) and protein production in adjacent fluids (GCF).

CHAPTER 5: MATERIALS AND METHODS

Patient Population

This one year, randomized, and double-masked clinical trial was conducted from March 2019-December 2020. Fifty patients, who were receiving periodontal maintenance therapy were screened and identified by faculty and investigators involved in this study at the University of Nebraska Medical Center (UNMC) College of Dentistry (RR, AK, MC and HR). Assessment included a review of medical and dental histories and previous oral hygiene and periodontal charting. Inclusion criteria for the study included subjects between the ages of 40-85 years, a periodontal diagnosis of advanced chronic periodontitis (Stage III-IV, Grade B (Tonetti et al. 2018)), one quadrant with at least three posterior teeth and one 6-9 mm periodontal pocket with a history of BOP and no radiographic vertical bony defect ≥1.5 mm, overall good systemic health, and a history of regular PMT. Exclusion criteria consisted of subjects with systemic diseases that significantly affect periodontal inflammation and bone turnover (e.g., chronic use of steroids or non-steroidal anti-inflammatory drugs, estrogens, bisphosphonates, calcitonin, methotrexate, antibiotics, >325mg aspirin/day), surgical periodontal therapy within the past year, and pregnant or breast-feeding females. Patients who met the inclusion criteria had the protocol explained and had all questions answered prior to obtaining consent.

Figure 1: Study Design Flowchart



Data Collection

Clinical measures and gingival crevicular fluid (GCF) collection were collected by one of three calibrated dentists (RR, AK, and RH). During GCF collection, the experimental site was isolated with cotton rolls and gently dried with gauze. Supragingival plaque was removed from the test teeth with a dental explorer. For GCF collection, an absorbent paper strip (PerioPaper Strips, Oraflow, Hewlett, NY) was inserted into the facial and lingual/palatal sulcus of the experimental site (Figure 2). After 30 seconds, the paper strips were immediately placed into a sterile vial and frozen at -80°C until further analysis. Strips contaminated by blood were discarded and were retaken after two minutes. Next, supragingival plaque, recession, probing depths, and bleeding on probing (BOP) within 30 seconds were recorded on 6 sites (MF, F, DF, ML, L, DL) on the experimental tooth and adjacent tooth.

Treatment Protocol

After the clinical data and GCF collection was completed, the investigator involved with data collection left, and the surgical / drug application phase of treatment was completed by a single clinician (LK, MB) and assistants (MC, LA) not involved with clinical measurements as described previously (Jasa et al. 2020). Following administration of local anesthesia to the experimental site, a #12B blade was used to reflect both the facial and lingual/palatal papilla, including in the experimental 6-9 mm interproximal pocket. Interproximal soft tissue was removed to allow access to the root. To measure activation of gene markers of inflammation and bone turnover, an approximately 2x2x2 mm piece of the interproximal tissue was placed in a sterile viral of 1.5 mL RNA*later* solution (ThermoFisher Scientific, Waltham, MA) (Figure 3) and frozen at -20°C until further analysis. Scaling and root planing was performed interproximally on the test site and on the adjacent interproximal tooth surface. Verification of a clean and smooth

root surface was done using an 11/12 explorer and the Perioscope (Perioscopy Unit, Zest Dental Solutions, Carlsbad, CA) by the clinician (Figure 4).

After mechanical therapy was completed, the clinician (LK or MB) randomly assigned patients to test simvastatin in methylcellulose (SIM/MCL) or control (MCL) groups. The root surface was etched for 2 minutes with ethylenediaminetetraacetic acid (EDTA, Pref-Gel, Straumann, Andover, MA) followed by irrigation with sterile saline. SIM and MCL were prepared by local compounding pharmacy (Pharmacy Solutions Lincoln, NE) and mixed immediately prior placement to achieve 2.2 mg simvastatin (SIM) suspended in 0.15 ml methylcellulose gel (MCL) (test group) or 0.15 ml of methylcellulose gel alone (control group)(Figure 5).

Gels were placed at the base of the pocket and deposited up the interproximal root surface of the experimental tooth (Figure 6). The papillae were re-approximated under pressure and sealed using cyanacrylate (PeriAcryl, Glustitch, Delta, BC, Canada). Routine periodontal maintenance therapy (PMT) then was completed by MC or LA, including full mouth periodontal charting, debridement, and root planing of inflamed pockets avoiding the experimental area. Patients were instructed to avoid brushing and flossing of the experimental site for 6 weeks. They were dispensed Listerine (Johnson & Johnson Consumer, Inc., New Brunswick, New Jersey) and instructed to be used twice a day for 30 seconds for 6 weeks. Patients were asked to return for postoperative visits after 2 and 6 weeks along with PMT recalls at 3, 6, 9, and 12 months. GCF collection was repeated at 2- weeks and 12-month PMT visits and clinical measurements were repeated at 12 months (Figure 7) by one of the three calibrated examiners (AK, RR, and HR). Collection of approximately 2x2x2 mm interproximal tissue was repeated at 2 weeks by either LK or MB. During the 6-week follow up, patients were given Gel-Kam® preventative treatment gel (Colgate Oral Pharmaceuticals, NY, NY) and a GUM® Proxabrush® (Sunstar

Americas, Inc., Schaumburg, IL) and patients were instructed to brush the experimental site twice a day using the provided interproximal brush. Participants were questioned about adverse events at 2-weeks and 6-months, and 12-months PMT visits.

Figure 2: Baseline GCF Sample

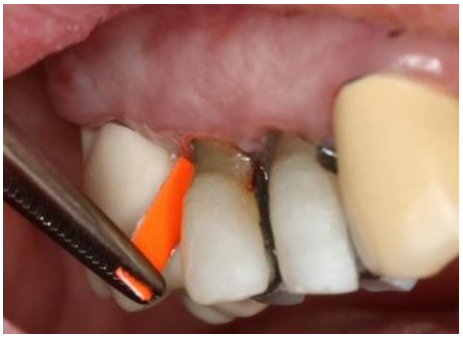


Figure 3: Baseline Interproximal tissue

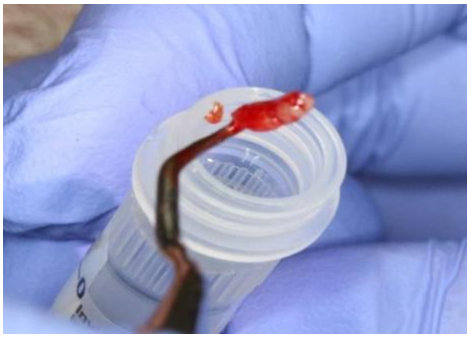


Figure 4: Perioscope use

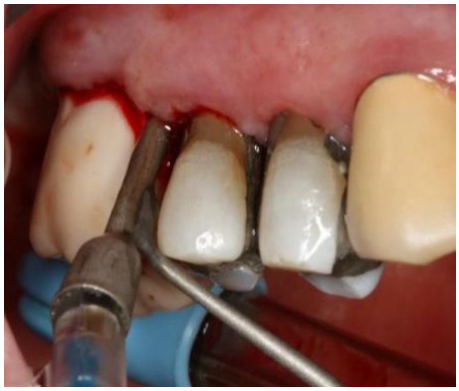


Figure 5: Mixing SIM & MCL

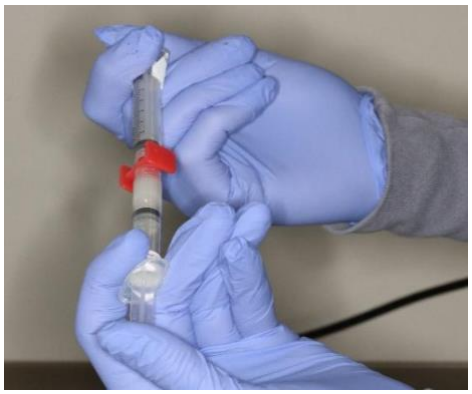


Figure 6: Placement of SIM

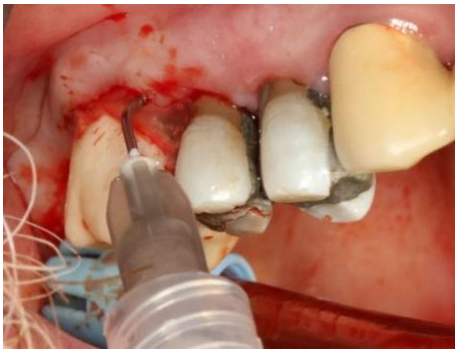
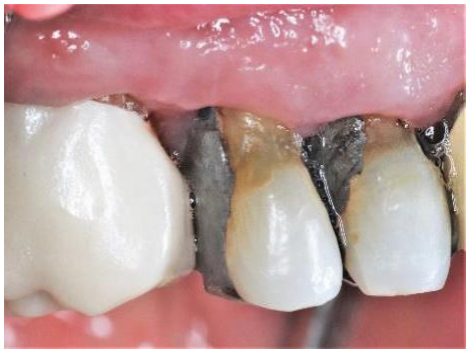


Figure 7: 12-months post-therapy



Analysis of GCF samples

Each GCF sample containing two paper strips was eluted using 85 µl of 1× PBS by gently agitating the samples on a rocker plate for 1 h at 4°C. Analyte concentrations were measured using magnetic bead panels (Millipore, Billerica, MA) and read on a MAGPIX with Luminex xPONENT software (Luminex Corporation, Austin, TX) per the manufacturers' recommendations.

Nine analytes were measured: Fibroblast growth factor (FGF-2), interleukin (IL)-1β, IL-6, IL-10, IL-17A, insulin-like growth factor 1 (IGF-1), tumor necrosis factor (TNF)-α, receptor activator of nuclear factor kappa-B ligand (RANKL), and vascular endothelial growth factor A (VEGF-A). The amounts of cytokines are reported in picograms per ml, then mathematically adjusted by multiplying 0.085 to achieve pg per sample. Gingival crevicular fluid (GCF), an exudate that can be harvested non-invasively from the gingival sulcus or periodontal pocket, contains a rich array of cellular and biochemical mediators that reflect the metabolic status of periodontal tissues.

Analysis of interproximal tissue samples

Interproximal tissue samples were collected between the interproximal sulcus of the experimental site and adjacent tooth and were stored in 1.5 mL RNA*later* solution (ThermoFisher Scientific, Waltham, MA). RNA extraction was conducted using the NucleoSpin RNA XS—complete kit for isolation and purification of total RNA from extremely small samples (Marcherey – Nagel, Düren, Germany). DNA digestion and cDNA synthesis were done using the QuantiTect Reverse Transcription Kit from QIAGEN© (Germantown, MD) per the manufacturers' recommendations.

Samples were diluted with water and placed in 96-well custom array plates in technical triplicate; qPCR was executed with SsoAdvanced Universal SYBR Green Supermix from Bio-Rad

(Hercules, CA) reagents. PCR conditions were 39 cycles at 95°C for 3 minutes (1 cycle) 95°C for 15 seconds, and at 58°C for 30 seconds (39 cycles). At the end point of rt-PCR analysis, the threshold cycle or ct vaules were recorded. To analyze the relative changes in gene expression, the 2^ΔΔct method was done, as described by Livak et al (2001).

Statistical analyses

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 deviation 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 most relevant previous studies (Killeen et al., 2012 and Jasa et al., 2019).

For the experimental interproximal treatment sulcus, BOP was considered present at baseline if at least one buccal or lingual interproximal site had the condition present. The follow-up variables for BOP were 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. Associations between categorical variables were assessed using Chi-Square tests, or Fisher's exact tests when expected cell counts were low. 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/MCL or MCL) for baseline BOP values. For the change in BOP

outcome, logistic regression models were used, which included group and adjusted for worst side. Adjusted odds ratios are presented with 95% confidence intervals.

Descriptive statistics for raw GCF and RT-PCR continuous data are given as medians and interquartile ranges (IQRs, representing the range of the middle 50% of the data). For analysis, data was log transformed due to skew.

General estimating equations were run with each measure of interest as the outcome, and each model included the variables (analyte) group and time, as well as the interaction between group and time to assess if change over time differed by group. If the interaction was not significant, main effects models were run. P-values for post hoc comparisons were adjusted using simulation methods. Pearson correlations were also run to assess for association between GCF and interproximal tissue samples at a given time point. Clinical attachment level (CAL) change between baseline and 12-months was the primary clinical outcome measure for this clinical trial. For change in CAL baseline CAL values were subtracted from 12 months CAL values, and difference scores were plotted against GCF and rt-PCR measures at two weeks (i.e. negative values indicate reduction in clinical attachment loss). Associations between change in CAL and measures at 2-weeks were assessed using Pearson correlations. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

CHAPTER 6: RESULTS

Patient Characteristics:

Following screening of the patients (Figure 1), 50 eligible patients were enrolled in the study. Intervention was initiated on all 50 subjects and 48 completed the 12-month PMT (4% dropout rate, one being from the test group and one from the control group). One patient did not return for the 9-month PTM. The other patient was diagnosed with lung cancer and only completed up to 6-month PMT. Reasons that patients dropped out were not believed to be related to the therapy administered.

Baseline characteristics of patients included in the study are represented in Table 1. There were no significant differences between groups at baseline in regards to age (p=0.76), gender (p=0.64), but smoking status was significant (p=0.03), with more smokers in the treat group (8 to 1).

Table 1: Demographics between groups

Variable	Simvastatin		Control		p-Value
Number	n=27	54%	n=23	46%	
Female	10	50.0	10	50.0	0.64††
Male	17	56.7	13	43.3	
Smoker	8	30%	1	4%	0.03^
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

Clinical outcomes:

The change in BOP from baseline to 12-month post therapy BOP measurements of experimental-tooth (interproximal) BOP are presented in Table 2.

Table 2: Change in BOP on Experiment Tooth Interproximal (Improvement/Maintained no BOP)

Group	**Tooth Surface**	**Adjusted^ Odds Ratio**	**95% Confidence Interval**		**P-Value for difference in change between groups**
SIM+MCL	Experimental Tooth	4.17	1.02	17.04	0.047*
MCL	Interproximal	1.00	Reference		
SIM+MCL	Adjacent Tooth	1.53	0.35	6.72	0.57
MCL	Interproximal	1.00	Reference		

^Models adjust for worst side
***Indicates significant difference**

The change in BOP from baseline to 12-month post-therapy BOP of experimental teeth was statistically significant for the test group (p=0.047). Patients treated with PR/RP + SIM+MCL (test) had 4.17 (95% CI AOR: 1.02, 17.04) times the odds greater than the control (PR/RP + MCL) group of having a good BOP outcome (i.e. showing improvement or maintaining no BOP), as reported in Krell et al. 2021.

Inflammatory Biomarker Outcomes: GCF

GCF samples from 50 patients were analyzed. General estimating equations were run with each measure of interest as the outcome, and each model included the variables group and time, as well as the interaction between group and time to assess if change over time differed by group. Comparisons for PR/RP+ SIM+MCL and PR/RP+ MCL taken at baseline, 2-weeks and 12-months post therapy are reported as follows:

The raw data of IL-6 are presented in Table 3a and the outcome model (after converting to pg/sample and log transformation) of IL-6 from baseline, 2-week, and to 12-month post therapy are presented in Table 3b. After adjusting for time, there was no significant difference between groups SIM+MCL and MCL (p=0.82) levels of IL-6. However, after adjusting for group, there was a significant difference (p<0.0001) in IL-6 between time points (Table 3b). Specifically, time two (2-weeks) was significantly higher than baseline and 12-months (p<0.0001).

Table 3a: IL-6 Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL	baseline	1.76	1.18/4.82
	2-week	11.18	2.00/184.82
	12-month	2.47	1.53/2.47
MCL			
	baseline	2.47	1.53/4.47
	2-week	7.29	2.24/78.00
	12-month	1.76	1.41/2.59

Table 3b: Outcome Model: Log IL-6

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.82		
	SIM+MCL	-1.01	0.16			
	MCL	-0.96	0.18			
Time				<.0001*		Adj. P value
	Baseline	-1.48	0.14		Baseline vs. 2 Weeks	<.0001*
	2 Weeks	0.14	0.31		2 weeks vs. 12 months	<.0001*
	12 Months	-1.61	0.13		Baseline vs. 12 Months	0.65

* Indicates significant difference

The raw data of IL-10 are presented in Table 4a and the outcome model of IL-10 from baseline, 2-week, and to 12-month post therapy are presented in Table 4b. After adjusting for time, there were no significant differences between groups SIM+MCL and MCL (p=0.30). After adjusting for group, there were no significant differences between time points (p=0.79) levels of IL-10 (Table 4b).

Table 4a: IL-10 Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL	baseline	7.76	0.38/14.12
	2-week	8.82	4.47/23.41
	12-month	7.53	4.24/13.41
MCL			
	baseline	8.12	5.29/13.76
	2-week	8.00	3.65/12.12
	12-month	6.82	4.00/8.71

Table 4b: Outcome Model: Log IL-10

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.30		
	SIM+MCL	-0.31	0.12			
	MCL	-0.49	0.13			
Time				0.79		Adj P value
	Baseline	-0.40	0.12		Baseline vs. 2 Weeks	0.90
	2 Weeks	-0.34	0.12		2 weeks vs. 12 months	0.77
	12 Months	-0.44	0.12		Baseline vs. 12 Months	0.96

* Indicates significant difference

However, when comparing changes in clinical attachment level (CAL) to GCF IL-10, there was a statistical significance between 2-week GCF and the 12-month CAL (Table 5). An increase in IL-10 at two weeks was significantly associated with a reduction (improvement) in CAL at 12-months ($r = -0.32$, $p = 0.03$).

Table 5: IL-10: Change in CAL to GCF

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations	
	log_IL10_2
CAL_exp_chg Change in CAL Exp (12 month - Baseline)	-0.31957 0.0304* 46

* Indicates significant difference

The raw data of IL-17 are presented in Table 6a and the outcome model of IL-17 from baseline, 2-week, and to 12-month post-therapy are presented in Table 6b. Level of GCF IL-17 were often low or undetectable. After adjusting for time, SIM+MCL group had significantly lower IL-17 than MCL alone (p=0.03) as reported in Table 6b). After adjusting for time, there was no significant difference over time between groups SIM+MCL and MCL alone (p=0.61).

Table 6a: IL-17 Raw Data

Group	**Time**	**Median (pg/ml)**	**25/75 Percentile**
SIM+MCL	baseline	0.24	0.24/0.35
	2-week	0.24	0.24/0.24
	12-month	0.24	0.24/0.24
MCL			
	baseline	0.24	0.24/1.76
	2-week	0.24	0.24/0.94
	12-month	0.24	0.24/0.82

Table 6b: Outcome Model: Log IL-17

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.03*		
	SIM+MCL	-3.59	0.13			
	MCL	-3.15	0.14			
Time				0.61		Adj P value
	Baseline	-3.32	0.13		Baseline vs. 2 Weeks	1.00
	2 Weeks	-3.33	0.13		2 weeks vs. 12 months	0.70
	12 Months	-3.46	0.13		Baseline vs. 12 Months	0.64

* Indicates significant difference

The raw data of RANKL are presented in Table 7a and the outcome model of RANKL from baseline, 2-week, and to 12-month post-therapy are presented in Table 7b. After adjusting for time, there was a trend towards less RANKL in SIM+MCL than MCL alone (p=0.06). After adjusting for group, there was no significant differences between time points (p=0.61).

Table 7a: RANKL Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL	baseline	1.41	0.35/4.47
	2-week	1.41	0.35/1.41
	12-month	1.41	0.35/2.12
MCL			
	baseline	1.41	1.41/3.88
	2-week	1.41	1.41/5.76
	12-month	1.41	1.41/5.56

Table 7b: Outcome Model: Log RANKL

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.06		
	SIM+MCL	-2.20	0.19			
	MCL	-1.66	0.21			
Time				0.61		Adj P value
	Baseline	-1.90	0.19		Baseline vs. 2 Weeks	0.61
	2 Weeks	-2.06	0.18		2 weeks vs. 12 months	0.61
	12 Months	-1.83	0.19		Baseline vs. 12 Months	0.91

* Indicates significant difference

The raw data of IGF-1 are presented in Table 8a and the outcome model of IGF-1 from baseline, 2-week, and to 12-month post therapy are presented in Table 8b. After adjusting for time, there was no significant differences between groups (p=0.34) and after adjusting for group, there was no significant differences between time points (p=0.67).

Table 8a: IGF-1 Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL	baseline	104.82	77.03/221.53
	2-week	121.06	77.06/165.41
	12-month	108.35	73.29/165.41
MCL			
	baseline	146.71	104.82/258.35
	2-week	143.06	79.76/183.88
	12-month	122.94	69.88/199.29

Table 8b: Outcome Model: Log IGF-1

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.34		
	SIM+MCL	2.26	0.11			
	MCL	2.41	0.12			
Time				0.67		Adj P value
	Baseline	2.39	0.10		Baseline vs. 2 Weeks	0.72
	2 Weeks	2.30	0.10		2 weeks vs. 12 months	1.00
	12 Months	2.30	0.10		Baseline vs. 12 Months	0.72

* Indicates significant difference

The raw data of FGF-2 are presented in Table 9a and the outcome model of FGF-2 from baseline, 2-week, and to 12-month post therapy are presented in Table 9b. After adjusting for time, there was no significant differences between groups (p=0.63) and after adjusting for group, there was no significant differences between time points (p=0.20).

Table 9a: FGF-2 Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL			
	baseline	12.00	11.88/41.06
	2-week	29.41	11.88/43.06
	12-month	33.53	11.88/49.65
MCL			
	baseline	28.24	11.88/42.47
	2-week	28.71	12.00/38.47
	12-month	25.06	5.41/43.06

Table 9b: Outcome Model: Log FGF-2

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.63		
	SIM+MCL	0.66	0.13			
	MCL	0.56	0.14			
Time				0.20		Adj P value
	Baseline	0.46	0.13		Baseline vs. 2 Weeks	0.18
	2 Weeks	0.72	0.13		2 weeks vs. 12 months	0.86
	12 Months	0.64	0.13		Baseline vs. 12 Months	0.43

* Indicates significant difference

The raw data of IL-1β are presented in Table 10a and the outcome model of IL-1β from baseline, 2-week, and to 12-month post-therapy are presented in Table 10b. After adjusting for time, there was no significant differences between groups (p=0.31) and after adjusting for group, there was no significant differences between time points (p=0.79).

Table 10a: Raw Data IL-1β

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL			
	baseline	88.94	31.18/213.76
	2-week	173.41	44.35/372.12
	12-month	83.06	35.29/259.18
MCL			
	baseline	103.65	52.59/884.24
	2-week	165.88	44.35/398.59
	12-month	71.53	28.35/313.53

Table 10b: GCF Outcome Model: Log IL-1β

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.31		
	SIM+MCL	2.08	0.23			
	MCL	2.43	0.25			
Time				0.79		Adj P value
	Baseline	2.24	0.23		Baseline vs. 2 Weeks	0.90
	2 Weeks	2.36	0.23		2 weeks vs. 12 months	0.78
	12 Months	2.16	0.24		Baseline vs. 12 Months	0.96

* Indicates significant difference

The raw data of TNF-α are presented in Table 11a and the outcome model of TNF-α from baseline, 2-week, and to 12-month post-therapy are presented in Table 11b. After adjusting for time, there was no significant difference between groups (p=0.28) and after adjusting for group, there was no significant differences (p=0.07). However, a trend toward an increase in TNF-α was seen at 2-weeks, particularly in SIM+MCL.

Table 11a: TNF-α Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL			
	baseline	6.94	3.76/17.29
	2-week	13.06	7.29/24.82
	12-month	9.06	7.29/17.88
MCL			
	baseline	9.29	5.41/14.12
	2-week	8.71	6.94/15.29
	12-month	8.00	2.71/11.88

Table 11b: Outcome Model: Log TNF-α

		Model Estimated Means	Standard Error	P-value	Post Hoc Comparisons	
Group				0.28		
	SIM+MCL	-0.21	0.12			
	MCL	-0.40	0.13			
Time				0.07		Adj P value
	Baseline	-0.44	0.13		Baseline vs. 2 Weeks	0.08
	2 Weeks	-0.08	0.13		2 weeks vs. 12 months	0.16
	12 Months	-0.39	0.13		Baseline vs. 12 Months	0.96

* Indicates significant difference

The raw data of VEGF-A are presented in Table 12a and the outcome model of VEGF-A from baseline, 2-week, and to 12-month post-therapy are presented in Table 12b. After adjusting for time, there was no significant difference in VEGF-a between groups ($p = 0.54$). After adjusting for group, there was a significant difference in VEGF-a between time points ($p=0.0002$). Specifically, 2-weeks was significantly higher than baseline ($p = 0.03$) and 12 months ($p < 0.0001$).

Table 12a: VEGF-A Raw Data

Group	Time	Median (pg/ml)	25/75 Percentile
SIM+MCL			
	baseline	49.88	28.00/94.24
	2-week	56.59	31.65/89.18
	12-month	42.00	20.00/60.71
MCL			
	baseline	59.06	20.47/80.59
	2-week	74.71	45.65/94.59
	12-month	35.65	24.94/46.94

Table 12b: Outcome Model: Log VEGF-A

		Model Estimated Means	**Standard Error**	**P-value**	**Post Hoc Comparisons**	
Group				0.54		
	SIM+MCL	1.28	0.12			
	MCL	1.38	0.13			
Time				0.0002*		Adj P value
	Baseline	1.25	0.16		Baseline vs. 2 Weeks	0.03
	2 Weeks	1.63	0.10		2 weeks vs. 12 months	<.0001*
	12 Months	1.11	0.10		Baseline vs. 12 Months	0.54

*Indicates a significant difference

Inflammatory Biomarker Outcomes: Interproximal Tissue Samples

One hundred (50 baseline and 50 2-week samples) samples were analyzed. General estimating equations were run with each measure of interest as the outcome, and each model included the variables group and time, as well as the interaction between group and time to assess if change over time differed by group. None of the models had a significant interaction, and only the raw data and the main effects were reported (Tables 13-18). None of the rt-PCR values varied significantly between groups or over time. Figures 8-13 represent raw rt-PCR data.

Table 13a: IL-6 Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	2.063573	1.632982	1.1396982	1.866044
Std Error	0.7642013	0.875252	0.3849045 3	0.614156
Std Dev	3.6649805	4.197561	1.7213453 7	3.070779

Figure 8: IL-6 Raw Data

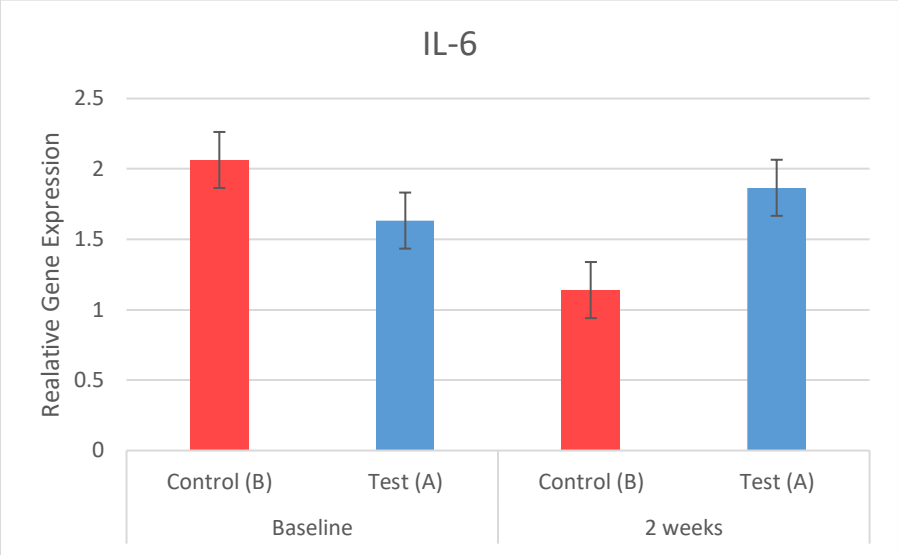


Table 13b: Interproximal Tissue Outcome Model: Log IL-6

		Model Estimated Means	**Standard Error**	**P-value**
Group				0.73
	SIM+MCL	-0.15	0.32	
	MCL	0.02	0.34	
Time				0.16
	Baseline	-0.39	0.33	
	2 Weeks	0.26	0.33	

IL-10 was higher in SIM+MCL, but no significant differences after log transformation (Table 14a and Figure 9).

Table 14a: IL-10 Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	1.868574	1.410128	1.411331	3.126981
Std Error	0.614232	0.459988	0.306459	1.112008
Std Dev	2.881003	2.253471	1.370526	5.549568

Figure 9: IL-10 Raw Data

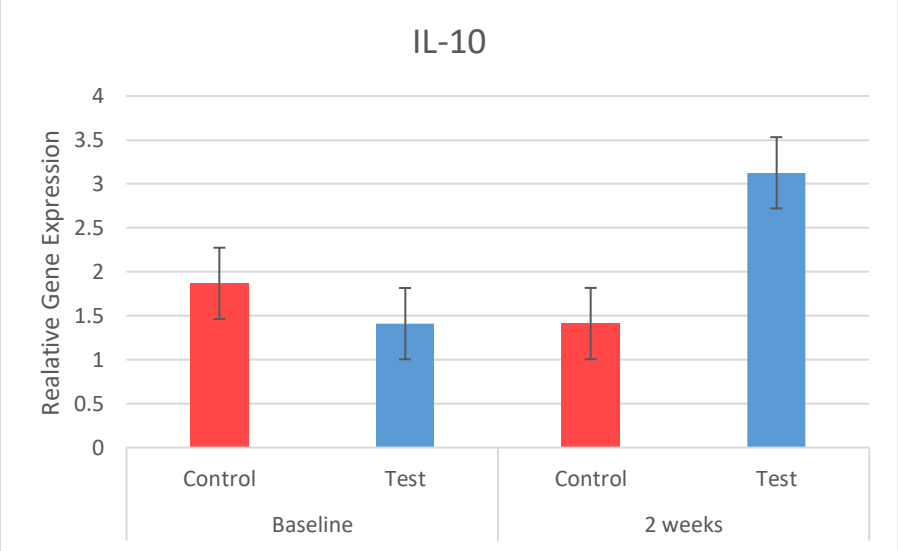


Table 14b: Interproximal Tissue Outcome Model: Log IL-10

		Model Estimated Means	**Standard Error**	**P-value**
Group				0.61
	SIM+MCL	0.20	0.24	
	MCL	0.02	0.27	
Time				0.12
	Baseline	-0.19	0.26	
	2 Weeks	0.40	0.26	

Table 15a: IL-17 Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	2.759067	0.581855	1.091678	49.67386
Std Error	1.030827	0.165972	0.392819	48.50159
Std Dev	4.493271	0.760581	1.756739	242.3161

Figure 10: IL-17 Raw Data

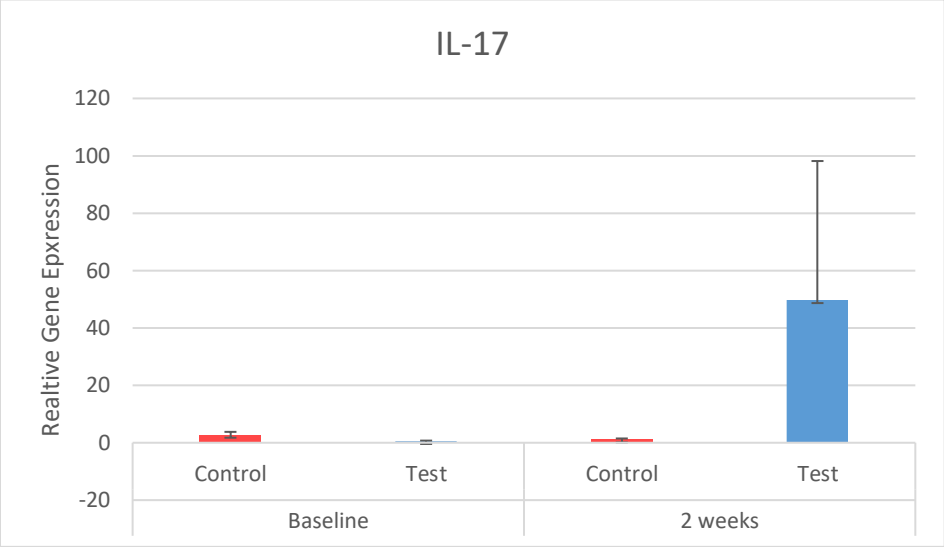


Table 15b: Interproximal Tissue Outcome Model: Log IL-17

		Model Estimated Means	**Standard Error**	**P-value**
Group				0.69
	SIM+MCL	-0.44	0.32	
	MCL	-0.25	0.36	
Time				0.70
	Baseline	-0.43	0.16	
	2 Weeks	-0.25	0.17	

RANKL was higher in SIM+MCL at 2-weeks, but no significance differences after log transformation (Table 16a and Figure 11).

Table 16a: RANKL Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	1.121855	0.600958	1.154585	2.626795
Std Error	0.171221	0.099079	0.196041	1.20613
Std Dev	0.784633	0.485388	0.898373	6.028717

Figure 11: RANKL Raw Data

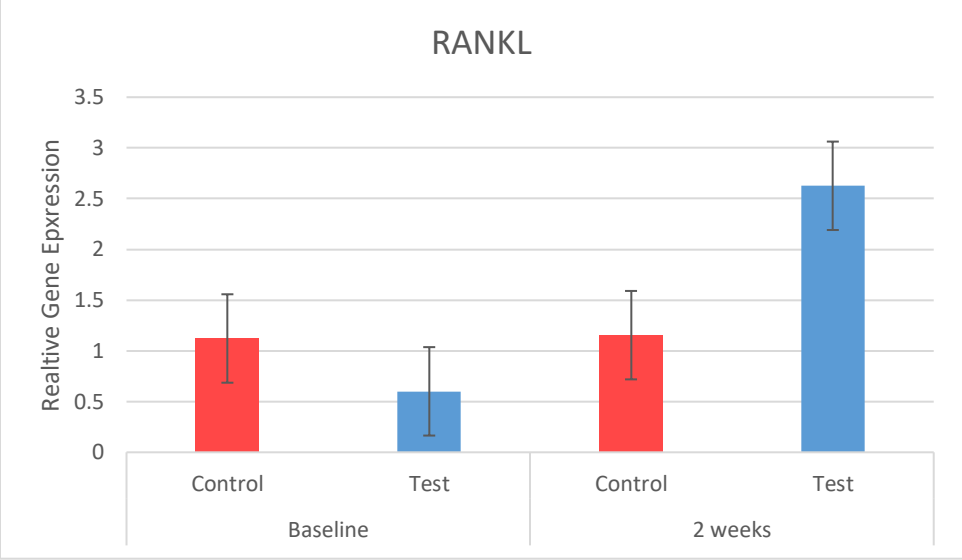


Table 16b: Interproximal Tissue Outcome Model: Log RANK-L

		Model Estimated Means	Standard Error	P-value
Group				0.84
	SIM+MCL	-0.06	0.16	
	MCL	-0.01	0.17	
Time				0.08
	Baseline	-0.28	0.18	
	2 Weeks	0.21	0.18	

IGF was greater in SIM+MCL at 2-weeks but no significance difference after log transformation (Table 17a and Figure 12).

Table 17a: IGF-1 Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	0.875368	0.88428	1.376801	1.855487
Std Error	0.207218	0.201438	0.4204	0.631547
Std Dev	0.97194	0.966063	1.880088	3.108066

Figure 12: IGF-1 Raw Data

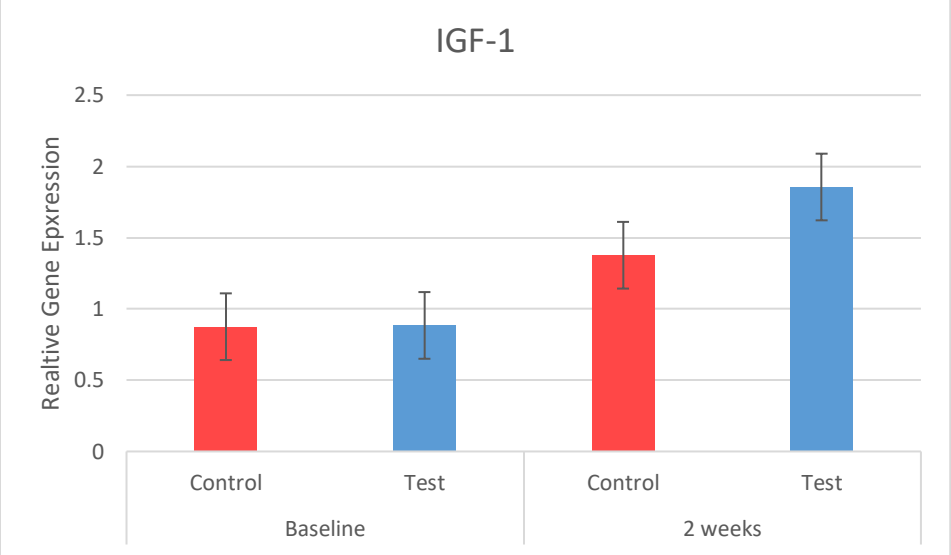


Table 17b: Interproximal Tissue Outcome Model: IGF-1

		Model Estimated Means	Standard Error	P-value
Group				0.98
	SIM+MCL	0.02	0.23	
	MCL	0.03	0.25	
Time				0.20
	Baseline	-0.17	0.22	
	2 Weeks	0.21	0.22	

Table 18a: FGF-2 Raw Data

	Baseline		2 weeks	
	Control	Test	Control	Test
Avg. Gene Exp	0.935285	0.801215	0.900269	1.020054
Std Error	0.197354	0.111739	0.172248	0.320358
Std Dev	0.904389	0.547406	0.789339	1.608924

Figure 13: FGF-2 Raw Data

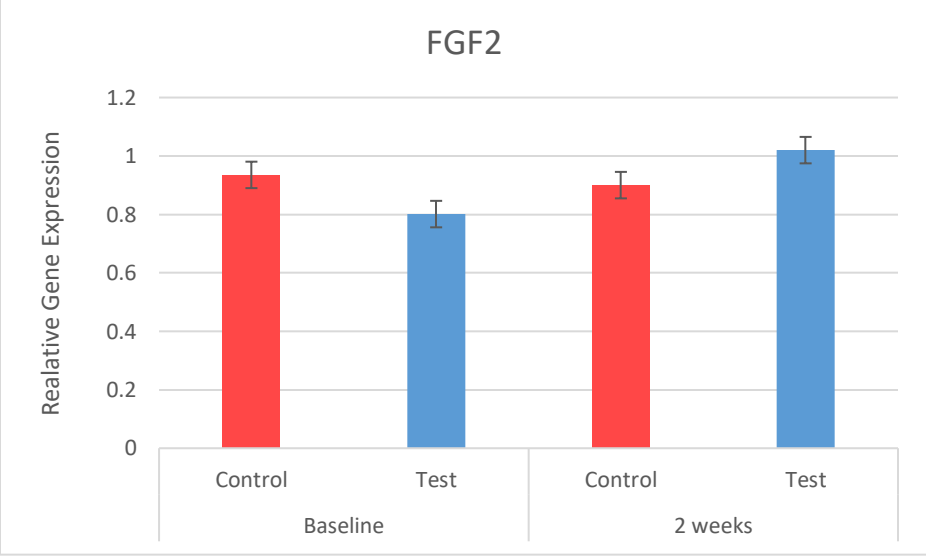


Table 18b: Interproximal Tissue Outcome Model: FGF-2

		Model Estimated Means	Standard Error	P-value
Group				0.92
	SIM+MCL	0.03	0.25	
	MCL	-0.01	0.27	
Time				0.17
	Baseline	-0.22	0.25	
	2 Weeks	0.24	0.24	

Pearson correlations were run to assess for association between GCF and the rt-PCR values. After adjusting for time and group, IL-6 was the only significant ($r = 0.33$, $p = 0.03$) interaction seen (Table 19).

Table 19: Pearson Correlation Coefficients – IL-6

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations		
	log_pIL6_0_FGE	log_gIL6_0
log_pIL6_0_FGE IL-6 2^-DDct (fold gene expression) baseline	1.00000 45	0.32798 0.0278 45
log_gIL6_0 IL6 baseline pg / 30 sec sample	0.32798 0.0278 45	1.00000 50

CHAPTER 7: DISCUSSION

This double-blinded, placebo-randomized controlled clinical trial aimed to compare inflammatory biomarker measurements of two therapies, PR/RP+ SIM/MCL (test) and PR/RP+MCL (control), for non-resolving 6-9 mm pockets in periodontal maintenance patients, at baseline, 2-weeks, and 12-months postoperatively. Patients included in this study were those who regularly attended a 3-month maintenance program. Precautions were taken to ensure that there was not any bias by compartmentalizing the various aspects of this study protocol as follows: masked examiners (RR, AK, RH) collecting all data and patients did not know group randomization. The clinicians (MB, LK) performed all papilla-reflections, scaling and root planing, and then randomization and placement of either SIM/MCL or MCL alone was performed.

The primary clinical outcomes measured in this study was change in clinical attachment levels, with secondary outcomes including changes in measures of inflammation: BOP after 12-months and inflammatory biomarkers (GCF [2-week and 12-month] and interproximal tissue at baseline and 2-weeks). The current study demonstrated no significant differences between groups at baseline in regard to age ($p=0.76$), gender ($p=0.64$), but more smokers were in the SIM+MCL group ($p=0.03$). Periodontal destruction is driven by an inflammatory response to bacterial biofilm. Bleeding on probing (BOP), suppuration, increased volume and inflammation markers in gingival crevicular fluid (GCF) are all indications of inflammation.

It is well known and studied that cigarette smoking is a major risk factor for periodontitis. The current study showed more smokers belonging to the SIM+MCL group (8 to 1). Kaldahl et al. (1996) concluded that heavy smokers (> 20 cigarettes per day) responded less favorably to periodontal treatment than light smokers. Giannopovlou et al. (1999) found that nicotine at high concentrations (100 ng/ml to 25 mg/nl) was cytotoxic and inhibited the

vacuolation and proliferation of fibroblasts. The current study had low numbers of smokers with majority of smokers in the SIM+MCL group. Smoking status may have impaired wound healing and decreased GCF levels and gene activation within the periodontal pocket. The number of cigarettes per day was not recorded in the current study.

When assessing change in BOP over time from baseline to 12-months, the current study showed that patients treated with PR/RP + SIM+MCL had 4.17 (95% CI AOR: 1.02, 17.04) times the odds of patients treated with PR/RP + MCL (control) of having a good BOP outcome (i.e. showing improvement or maintaining no BOP), ($p = 0.047$) as reported in Table 2. This positive outcome is supported by Joss et al. (1999), who stated that patients with a higher mean BOP have a higher risk for further attachment loss at single sites. Wilson et al. (2008) confirmed the presence of BOP indicates histological inflammation or presence of bacteria. Therefore, the presence of BOP could be an important clinically because it could aid as a prognostic tool for future BOP and periodontal attachment loss. Disagreeing with this statement, Chavez et al. (1990) stated that the presence of BOP is not a necessarily a reliable predictor of disease. One of the reasons for this includes the variability in pressure amongst clinicians when probing.

The current study is in line with previous studies (Pradeep et al. 2010, Pradeep et al. 2012, Pradeep et al. 2013, Rao et al. 2013, and Priyanka et al. 2017) reporting the use of SIM and the effects of SIM on clinical inflammation (BOP). Pradeep et al., (2012) showed a decreased gingival bleeding index from baseline to 6 months ($p=0.001$). Lindy et al. (2008) showed a similar effect of SIM in patients with chronic periodontitis who were on systemic statin therapy. Patients with periodontitis taking statin had a 37% few pathologic periodontal pockets than those not taking statin medication. Therefore, the authors all concluded that a decrease in gingival bleeding index (BOP) was due to the anti-inflammatory effects of SIM.

The clinical effects of SIM have been evaluated over the years; however, the effect SIM has on inflammatory biomarkers and growth factors specifically IL-6, IL-10, IL-17, IGF-1, TNF-α, RANKL, and VEGF-A is more limited.

IL-6 is a pro-inflammatory cytokine involved in the regulation of host response to tissue injury and infection. Excess amounts of IL-6 are produced locally in patients with chronic periodontitis (Bozkurt et al., 2000). In the current study, when evaluating IL-6 in GFC; after adjusting for time, there was no significant difference in IL-6 between groups ($p = 0.82$) as reported in Table 3b. However, after adjusting for group, there was a significant increase in IL-6 at 2-weeks in both groups (Table 3b). It should be noted that baseline IL-6 levels were low, possibly due to the low levels of inflammation that was seen in these patients receiving PMT. Then following PR/RP, an increase in pro-inflammatory markers (i.e. IL-6) is expected at 2-weeks. When comparing the current study to others, Gunjiganur Vemanaradhya et al. (2017) showed that GCF levels of IL-6 was reduced at 45 days post-operatively in the test group (1.2% SIM) compared to the control when 1.2 % SIM was delivered locally in intrabony defects as an adjunct to initial therapy (SRP). The current study was done during PMT and not as adjunct to initial therapy (SPR) as seen in Vemanaradhya et al. (2017), so changes between groups from low baseline levels may be more difficult to detect. GCF levels correlated with $r=0.33$ and $p=0.03$ (Table 19), suggesting that IL-6 gene activation may lead to protein production in the periodontium. This correlation was the only significant relationship between GCF and tissue rt-PCR, indicating that gene activation of the other markers did not reflect protein production in the surrounding fluids.

IL-10 is a potent anti-inflammatory cytokine that is produced by many different cell types. As an anti-inflammatory cytokine, IL-10 plays an important role in periodontal disease

(Zhang et al., 2014). In the current study, and after adjusting for time, there was no significant difference in IL-10 between groups ($p = 0.82$). After adjusting for group, there was no significant differences as reported in Table 4b. When looking at the relative gene expression for IL-10 (Table14a, Figure 9), levels of IL-10 found in the test group (PR-RP + SIM+MCL) were increased at two weeks. However, when compared with log transformed statistical analysis, no significant differences were reported. When evaluating CAL at 12-months and IL-10 comparisons, our study found that IL-10 GCF levels at two weeks were significantly associated with a reduction of CAL from baseline to 12 months ($r = -0.32$, $p = 0.03$) as reported in Table 5. These results could be explained to the anti-inflammatory effects SIM has on periodontal disease. When comparing to other studies, Glover et al. (2016) showed that when SIM was delivered subgingival in periodontal pockets, IL-10 is stimulated in the GCF and improves the periodontal attachment. Liu et al. (2019) reported that IL-10 is upregulated in SIM-treated rat molar periodontal tissues.

IL-17 is another pro-inflammatory cytokine that plays an important role in the pathogenesis of periodontal inflammation. IL-17 has been shown to stimulate production of various other cytokines (i.e. TNF-α, IL-6, IL-1β) and can also increase the expression of RANKL (Stadler et al. 2016). The research on IL-17 and the use of locally delivered SIM in humans is lacking. In the current study, after adjusting for time, the SIM+MCL group had significantly lower IL-17 GCF levels than group MCL alone ($p = 0.03$) as reported in Table 7a. However, the GCF IL-17 levels were very low at baseline (undetectable), making interpretation difficult. Liu et al. (2019) showed that when SIM is locally delivered in SIM-treated rat molar periodontal tissue that after 48 hours, IL-17 levels were decreased (3.55 fold decrease) when compared to baseline. These results could be explained by the anti-inflammatory effects SIM has on periodontal disease.

IGF-1 is growth factor hormone that has been shown to be a key player in periodontitis due to its potent bone anabolic factors that are responsible for limited periodontal destruction (Okada et al., 1998). The research on IGF-1 and the use of locally delivered SIM in humans is lacking. The current study found that neither the GCF levels nor the rt-PCR data showed any significant differences after log transformation was done. However, prior to log transformation, rt-PCR in SIM+MCL group was increased at 2-weeks when compared to MCL alone (Table 17a, Figure 12). Liu et al. (2019) reported for the first time showing an association between SIM treatment and the upregulation IGF-1 in experimental periodontitis. IGF-1 levels were significantly ($p<0.001$) elevated expression in response to SIM treatment compared to the controls (Liu et al., 2019). However, no significant differences between groups or over time found in GCF in the current study.

RANK-L is responsible for stimulation of osteoclast differentiation and bone resorption (Lacey et al., 1998). RANK-L is produced as a membrane-bound or secreted ligand by osteoblasts, fibroblasts, and activated T- and B-cells. The current study showed that after adjusting for time, there was a trend toward less RANKL in GCF in the SIM+MCL group than MCL alone ($p=0.06$) as reported in Table 7b. Both rt-PCR groups (SIM+MCL and MCL) were trending ($p=0.08$) at 2-weeks (TABLE 16b). rt-PCR in the SIM+MCL group was increased at 2-weeks but not significant after log transformation (Table 16a Figure 11). Suthanthiran et al. (2019) reported the use of a SIM-loaded collagen membrane in the treatment of intrabony defects in patients with chronic periodontitis. Suthanthiran et al. (2019) showed RANKL GCF levels were decreased significantly ($p<0.001$) in test subjects (SIM-loaded collagen membrane) when compared to control (membrane alone) after 21 days. Another study by Ayukawa et al. (2009) investigated whether or not the use of locally delivered SIM affected both the cellular events and bone formation in bone created defects in rats. Ayukawa et al. (2009) concluded that after

5 days, the statin group demonstrated significantly larger new bone when compared to the controls. Although the levels of both RANK and OPG were not affected by SIM, the expression of RANKL was depressed (Ayukawa et al. (2009). The above studies both have reported decreased levels of RANKL, furthering explaining that osteoclast suppression may be the consequence of RANKL depression. However, short-term human gene levels were elevated at 2-weeks, perhaps due to the increased bone turnover proceeding the anabolic stage of bone growth. Unfortunately. RANKL levels in current GCF samples were low and often undetectable, and no bone growth was noted clinically (Krell et al. 2021).

TNF-α is a potent osteoclastogenic cytokine that is produced during an inflammatory response (Lam et al., 2000). As a pro-inflammatory cytokine, TNF- α plays an important role in the progression of periodontitis. In the current study, after adjusting for time, there were no significant differences between groups ($p=0.28$) and after adjusting for group, there were no significant differences ($p=0.07$). However, a trend ($p=0.07$) toward an increase in TNF-α in both groups, particularly SIM+MCL was seen at 2-weeks (Table 11b). The increase of TNF-α could be explained due to the localized wound healing response. Bahammam et al. (2018) looked at the relationship between levels of IL-6 and TNF-α, and SIM usage in GCF of diabetic patients with chronic periodontitis. Bahammam et al. (2018) concluded that increased levels of IL-6, and TNF-α GCF were seen in diabetic patients with chronic periodontitis. However, decreased levels of IL-6 and TNF-α were seen in the group who had been using 20 mg SIM as lipid-lowering agent between 5 and 10 years. Although Bahammam et al. (2018) study design was different than the current study, Bahamman et al. (2018) showed the effects of SIM has not only on lowering systemic inflammation but also the potential SIM has on lowering inflammation (IL-6 and TNF-α) within the periodontal pocket.

Vascular endothelial growth factor A (VEGF-A) is a multifunctional angiogenic cytokine that plays an important role in inflammation and wound healing. After adjusting for time, there was no significant difference in VEGF-a between groups ($p = 0.54$). After adjusting for group, there was a significant difference in VEGF-a between time points ($p=0.0002$). Specifically, 2-weeks was significantly higher than baseline ($p = 0.03$) and 12 months ($p < 0.0001$) as reported in Table 12b. The relationship between GCF levels of VEGF-A and periodontitis is well studied (Booth et al., 1998, Johnson et al. 1999, Prapulla et al. 2007, Pradeep et al. 2011). Booth et al. (1998) examined patients with chronic periodontitis and the relationship of VEGF within GCF samples. The volume of GCF and total amount of VEGF were greater in diseased sites compared to clinically healthy sites. Johnson et al. (1999) showed that VEGF and IL-6 concentrations were significantly lower within healthy gingiva than within diseased sites, and the number of blood vessel profiles and mean IL-6 concentrations were highest in diseased sites. A study done by Prapulla et al. (2007), concluded that VEGF concentration in GCF increases proportionally with the progression of periodontitis. Pradeep et al. (2011), showed the relationship between GCF VEGF-A levels in periodontally healthy patients. GCF and serum VEGF levels increased progressively (6-8 weeks post-therapy) with the disease severity and decreased after periodontal treatment (Pradeep et al. 2011). All the previous studies indicate the key role VEGF plays in periodontal disease as an inflammatory biomarker.

SIM+MCL as an adjunct to SRP has been shown to improve CAL and BOP, particularly as part of initial therapy (Pradeep et al., 2010, Pradeep et al. 2012, Amrosio et al., 2018, Muniz et al., 2018). Unfortunately, SIM/MCL is not commercially available in the United States, and its use during PMT is lacking. A major difference between the current study and previous studies that they reported the effect of SIM has during initial therapy (SRP) and not being maintained by regular PMT. Whereas, the current study, to the best of our knowledge is the first clinical trial in

the United States that studied the effects of SIM during PMT. In addition, no previous studies have evaluated this bordered spectrum of cytokines and growth factors in response to local SIM application in humans, particularly including short- and long-term results.

There are several different limitations in this study that should be addressed. Firstly, this study was conducted during the COVID pandemic, causing some delay in PMT appointments and 12-month sampling. Patients were observed for a period of only 12-months and a longer duration would be ideal to determine long term stability. This may have influenced the response to the treatment and inflammatory condition of the pockets. Although a control was used in the current study, both treatment modalities incorporated the use of the endoscope. The enhanced visualization provided by the endoscope may have reduced the effect which would have been seen between the two treatments due to the superior root debridement that is allowed.

Additional studies could look more closely at the way SIM affects other possible cytokines, growth factors, and inflammatory cells. If key inflammation cells are present, therapies can be introduced to help control the response, and in turn improve the clinical outcome.

CHAPTER 8: CONCLUSION

Scaling and root planing with papilla reflection in inflamed, persistent, deep periodontal pockets during PMT with the addition of SIM, resulted in clinical improvements in BOP after 12-

months. Increase in 2-week GCF IL-10 was correlated with improved CAL. Short-term wound healing resulted in increased GCF IL-6 and VEGF, which decreased after 12-months as clinical outcomes improved, suggesting early wound healing inflammatory markers may play an important role in the healing phase.

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APPENDIX A: RAW 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

APPENDIX B: 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			

APPENDIX C: GCF RAW CLINICAL DATA - FGF-2

Patient	Group	FGF2 Baseline pg/30 sec sample	FGF2 2 week pg/30 sec sample	FGF2 12month pg/30 sec sample
1	B	0.92	0.43	0.43
2	B	0.43	2.58	0.43
3	A	1.43	2.79	2.58
4	B	0.92	0.43	2.34
5	B	0.43	2.22	2.99
6	B	2.08	3.08	1.43
7	B	2.08	2.79	0.46
8	B	2.69	3.18	
9	B	0.43	1.62	0.43
10	A	0.43	1.07	2.15
11	A	0.43	2.46	5.68
12	A	0.43	0.43	1.20
13	B	2.58	3.27	1.32
14	A	0.43	2.58	0.43
15	A	2.34	0.43	2.99
16	A	3.08	0.43	1.79
17	A	4.68	5.85	3.92
18	B	2.69	1.43	3.35
19	B	3.61	4.48	
20	B	2.40	0.43	0.43
21	B	2.46	2.34	0.92
22	A	0.43	1.79	0.43
23	A	1.94	3.18	1.94
24	A	0.43	0.43	0.43
25	B	2.08	0.43	0.43
26	B	4.09	4.46	4.88
27	A	4.78	16.23	1.01
28	A	1.01	6.40	3.66
29	A	3.66	2.55	4.88
30	A	3.81	2.13	2.85
31	A	3.49	3.66	4.22
32	A	1.02	16.34	2.85
33	A	1.01	3.09	5.07
34	A	3.66	2.55	4.57
35	B	3.66	1.01	3.96
36	A	1.01	1.01	1.01

37	A	1.01	1.01	1.01
38	A	4.09	19.43	
39	A	1.01	1.01	4.09
40	A	3.09	3.96	4.57
41	A	3.49		2.85
42	A	1.01	1.01	1.01
43	A	1.01	2.13	4.78
44	B	3.96	3.49	3.49
45	B	1.01	1.02	3.96
46	B	4.34		4.22
47	B	3.66	2.55	1.01
48	B	1.01	3.49	3.66
49	B	2.55	4.98	2.13
50	B	1.01	2.13	3.66
	A = SIM+MCL			
	B= MCL			

APPENDIX D: GCF RAW CLINICAL DATA – IL-1β

Patient	Group	IL-1β baseline pg/30 sec sample	IL-1β 2 week pg/30 sec sample	IL-1β 2 week pg/30 sec sample
1	B	1.86	15.22	6.33
2	B	5.41	3.68	1.70
3	A	26.38	21.54	140.08
4	B	1.47	0.60	2.08
5	B	3.93	7.75	26.65
6	B	106.77	27.87	3.15
7	B	8.82	12.98	13.09
8	B	263.12	7.42	
9	B	3.94	3.66	0.27
10	A	9.39	4.41	11.08
11	A	8.72	34.35	2.40
12	A	2.65	0.52	3.68
13	B	34.62	23.75	2.41
14	A	18.53	87.03	4.03
15	A	8.35	1.11	22.03
16	A	18.17	3.77	17.29
17	A	3.59	6.19	4.96
18	B	8.81	4.36	2.63
19	B	28.76	64.81	
20	B	21.75	5.66	2.75
21	B	4.47	22.29	1.00
22	A	7.07	23.90	17.82
23	A	12.98	17.74	143.15
24	A	3.20	1.14	1.86
25	B	743.17	62.01	0.62
26	B	119.71	23.93	167.47
27	A	30.06	40.86	0.82
28	A	2.20	23.19	3.00
29	A	25.20	3.82	19.03
30	A	2.14	11.75	5.21
31	A	10.77	17.75	7.06
32	A	0.80	70.64	6.99
33	A	7.56	141.80	103.06
34	A	51.53	28.74	184.24
35	B	6.92	3.77	30.00
36	A	2.09	1.02	0.58

37	A	1.95	4.85	
38	A	13.35	55.89	
39	A	0.03	0.60	2.97
40	A	6.31	31.63	26.60
41	A	42.10		22.31
42	A	2.89	4.55	1.54
43	A	3.09	1.02	16.38
44	B	140.40	36.32	6.08
45	B	6.18	33.88	4.63
46	B	75.16		46.33
47	B	30.76	3.15	7.91
48	B	8.80	38.90	288.76
49	B	6.74	2.06	61.65
50	B	1.00	53.44	13.37
	A = SIM+MCL			
	B= MCL			

APPENDIX E: GCF RAW CLINICAL DATA – IL-6

Patient	Group	IL-6 baseline pg / 30 sec sample	IL-6 2 week pg / 30 sec sample	IL-6 12 month pg / 30 sec sample
1	B	0.10	0.67	0.07
2	B	0.05	0.19	0.09
3	A	0.21	6.71	1.32
4	B	0.13	0.06	0.15
5	B	0.05	0.18	0.13
6	B	0.63	0.57	0.18
7	B	0.25	1.79	1.10
8	B	0.24	4.75	
9	B	0.45	0.11	0.06
10	A	1.10	0.36	0.26
11	A	1.15	1.49	0.37
12	A	0.15	0.06	0.13
13	B	0.87	0.19	0.11
14	A	0.33	0.64	0.14
15	A	0.10	0.04	0.07
16	A	0.14	0.14	0.15
17	A	0.09	26.90	0.17
18	B	0.15	21.73	0.42
19	B	0.16	30.30	
20	B	0.15	13.88	0.12
21	B	0.38	1.95	0.12
22	A	0.06	1.54	0.20
23	A	0.22	35.35	1.47
24	A	1.93	0.08	0.42
25	B	0.19	0.50	0.14
26	B	0.32	0.38	0.44
27	A	0.25	32.57	0.09
28	A	0.10	40.67	0.11
29	A	0.08	0.93	0.15
30	A	0.13	0.17	0.11
31	A	0.08	0.84	0.14
32	A	0.41	75.68	0.88
33	A	0.14	15.71	0.16
34	A	0.09	0.10	0.19
35	B	8.07	0.12	3.00
36	A	0.75	0.05	0.06
37	A	0.14	6.34	0.16

38	A	0.44	24.67	
39	A	0.05	0.45	0.12
40	A	0.27	0.99	0.16
41	A	0.26	0.00	0.13
42	A	0.61	0.33	0.21
43	A	0.12	0.96	0.13
44	B	0.30	0.43	0.15
45	B	0.12	0.67	0.94
46	B	0.99	0.00	0.16
47	B	0.31	6.63	0.13
48	B	0.21	8.05	0.16
49	B	0.14	8.58	0.11
50	B	0.08	0.13	0.22
	A = SIM+MCL			
	B= MCL			

APPENDIX F: GCF RAW CLINICAL DATA – IL-10

Patient	Group	IL 10 baseline pg / 30 sec sample	IL10 2 week pg / 30 sec sample	IL10 12 month pg / 30 sec sample
1	B	0.25	0.28	0.21
2	B	0.50	0.60	0.42
3	A	0.48	0.44	1.07
4	B	0.25	0.17	0.58
5	B	0.17	0.21	1.08
6	B	0.63	0.64	0.30
7	B	0.56	1.03	0.52
8	B	0.54	0.31	0.00
9	B	1.00	0.59	0.36
10	A	1.63	4.54	2.61
11	A	1.20	0.58	2.39
12	A	0.80	0.52	1.03
13	B	0.81	0.83	0.34
14	A	0.60	0.38	0.73
15	A	0.23	0.17	0.68
16	A	0.75	0.73	2.17
17	A	0.66	0.23	0.66
18	B	0.30	1.39	0.74
19	B	0.69	1.03	
20	B	1.17	0.73	1.15
21	B	0.48	0.77	0.28
22	A	0.62	5.81	7.38
23	A	0.42	2.67	4.32
24	A	3.52	1.03	1.14
25	B	1.27	5.64	0.17
26	B	1.27	1.29	0.69
27	A	1.64	2.20	0.43
28	A	1.54	1.99	0.27
29	A	0.31	0.56	0.40
30	A	0.54	0.69	0.36
31	A	0.22	0.77	0.36
32	A	3.71	2.36	0.45
33	A	0.22	0.36	0.90
34	A	0.38	2.05	1.93
35	B	1.19	0.47	0.69
36	A	0.75	0.21	0.21

37	A	0.27	0.94	0.24
38	A	1.17	0.98	
39	A	0.21	0.21	0.34
40	A	3.09	0.81	0.62
41	A	0.75		0.31
42	A	0.67	1.00	0.45
43	A	0.38	0.21	0.36
44	B	1.17	0.29	0.21
45	B	1.25	0.77	1.48
46	B	0.90		2.05
47	B	3.61	1.02	1.40
48	B	0.69	1.09	0.69
49	B	0.45	0.45	0.36
50	B	0.21	0.24	0.62
	A = SIM+MCL			
	B= MCL			

APPENDIX G: GCF RAW CLINICAL DATA – IL-17a

Patient	Group	IL17 Baseline pg / 30 sec sample	IL-17 2 week pg / 30 sec sample	IL-17 12month pg / 30sec sample
1	B	0.02	0.02	0.02
2	B	0.02	0.08	0.02
3	A	0.02	0.02	0.02
4	B	0.07	0.02	0.02
5	B	0.02	0.02	0.03
6	B	0.20	0.11	0.02
7	B	0.22	0.15	0.14
8	B	0.15	0.05	
9	B	0.02	0.02	0.02
10	A	0.02	0.02	0.02
11	A	0.10	0.02	0.02
12	A	0.02	0.02	0.02
13	B	0.14	0.18	0.02
14	A	0.02	0.02	0.02
15	A	0.03	0.02	0.13
16	A	0.03	0.02	0.02
17	A	0.02	0.08	0.02
18	B	0.05	0.07	0.13
19	B	0.02	0.24	
20	B	0.02	0.02	0.02
21	B	0.02	0.02	0.02
22	A	0.02	0.02	0.02
23	A	0.03	0.02	0.15
24	A	0.02	0.02	0.02
25	B	0.08	0.07	0.02
26	B	0.24	0.19	0.37
27	A	0.35	0.79	0.02
28	A	0.02	0.07	0.02
29	A	0.05	0.02	0.02
30	A	0.02	0.02	0.02
31	A	0.02	0.02	0.02
32	A	0.02	0.02	0.02
33	A	0.02	0.02	0.21
34	A	0.02	0.02	0.02
35	B	0.02	0.02	0.21
36	A	0.02	0.02	0.02

37	A	0.02	0.02	0.02
38	A	0.02	0.07	
39	A	0.02	0.02	0.02
40	A	0.02	0.41	0.02
41	A	0.10		0.02
42	A	0.02	0.02	0.02
43	A	0.02	0.02	0.10
44	B	0.19	0.02	0.02
45	B	0.02	0.02	0.02
46	B	0.45	0.00	0.26
47	B	0.07	0.02	0.02
48	B	0.02	0.07	0.07
49	B	0.02	0.02	0.02
50	B	0.02	0.02	0.02
	A = SIM+MCL			
	B= MCL			

APPENDIX H: GCF RAW CLINICAL DATA – IGF1

Patient	Group	IGF1 baseline pg / 30 sec sample	IGF1 2 week pg/30 sec sample	IGF1 12 month pg/30 sec sample
1	B	13.74	5.94	14.24
2	B	15.89	13.99	15.00
3	A	10.70	24.08	46.81
4	B	2.64	3.44	8.65
5	B	9.42	10.96	34.45
6	B	19.86	6.78	5.22
7	B	21.96	13.99	14.49
8	B	32.71	19.86	0.00
9	B	15.51	3.44	9.17
10	A	7.05	6.23	7.32
11	A	20.12	15.63	18.06
12	A	8.91	5.94	6.23
13	B	21.96	13.74	10.45
14	A	9.81	10.70	18.96
15	A	7.86	4.45	9.17
16	A	3.06	2.20	6.23
17	A	7.59	11.97	5.94
18	B	10.96	13.23	9.42
19	B	12.47	15.63	
20	B	23.82	12.60	39.24
21	B	15.51	19.48	5.94
22	A	29.29	39.86	10.70
23	A	18.83	23.28	14.12
24	A	25.16	12.47	13.11
25	B	8.91	10.32	2.64
26	B	22.53	16.37	26.74
27	A	20.66	19.80	20.38
28	A	4.05	14.35	6.91
29	A	14.06	4.53	14.06
30	A	17.23	4.53	13.19
31	A	6.55	8.11	8.93
32	A	1.55	12.75	5.79
33	A	8.44	13.63	15.22
34	A	20.52	11.11	4.05
35	B	87.42	38.59	46.72
36	A	34.69	9.25	13.19
37	A	17.80	7.94	2.13

38	A	4.05	14.06	0.00
39	A	4.75	7.08	9.25
40	A	8.61	6.55	4.05
41	A	7.61		2.64
42	A	5.98	7.94	7.26
43	A	12.01	9.88	11.71
44	B	9.57	8.93	10.19
45	B	10.50	16.08	18.66
46	B	10.34		11.56
47	B	7.26	8.61	2.13
48	B	2.64	11.71	3.52
49	B	2.64	4.97	16.94
50	B	7.26	2.64	4.97
	A = SIM+MCL			
	B= MCL			

APPENDIX I: GCF RAW CLINICAL DATA – RANKL

Patient	Group	RANKL baseline pg / 30 sec sample	RANKL 2 week pg / 30 sec sample	RANKL 12month pg / 30 sec sample
1	B	0.12	0.12	0.12
2	B	0.12	0.12	0.38
3	A	0.12	0.12	0.12
4	B	0.12	0.12	0.12
5	B	0.59	0.49	0.59
6	B	0.97	0.12	0.12
7	B	0.38	0.12	0.12
8	B	1.66	0.12	
9	B	0.12	0.59	0.12
10	A	0.38	0.12	0.12
11	A	0.59	0.12	0.12
12	A	1.83	0.12	1.83
13	B	1.83	2.77	0.38
14	A	0.59	0.38	0.12
15	A	0.12	0.12	0.38
16	A	0.12	0.12	0.12
17	A	0.12	0.12	0.12
18	B	0.12	0.12	0.12
19	B	0.12	0.97	0.12
20	B	0.12	0.12	0.12
21	B	0.12	0.12	0.12
22	A	0.38	0.12	0.12
23	A	0.12	0.12	0.12
24	A	0.12	0.12	0.12
25	B	0.12	1.15	0.12
26	B	0.33	0.77	1.00
27	A	0.92	1.67	0.03
28	A	5.00	3.03	4.55
29	A	0.03	0.03	0.77
30	A	0.03	0.03	0.33
31	A	0.18	0.18	0.03
32	A	0.03	0.62	0.03
33	A	0.03	0.03	0.25
34	A	0.03	0.03	0.03
35	B	0.03	0.03	0.48
36	A	0.03	0.03	0.03
37	A	0.03	0.03	0.03

38	A	0.18	0.03	
39	A	0.03	0.03	0.03
40	A	0.03	0.03	0.03
41	A	0.18		0.18
42	A	0.18	0.18	0.03
43	A	0.03	0.03	0.03
44	B	0.33	0.03	1.37
45	B	0.33	0.03	5.76
46	B	0.03		0.03
47	B	0.33	0.18	1.37
48	B	0.03	0.18	0.18
49	B	0.03	0.03	0.03
50	B	0.03	0.03	0.03
	A = SIM+MCL			
	B= MCL			

APPENDIX J: GCF RAW CLINICAL DATA – TNF-α

Patient	Group	TNF-α baseline pg/30 sec sample	TNF-α 2 week pg/30 sec sample	TNF-α 12 month pg/30 sec sample
1	B	0.30	0.65	0.46
2	B	0.26	1.00	0.17
3	A	2.54	2.37	2.08
4	B	0.36	0.11	0.23
5	B	0.11	0.30	0.52
6	B	0.88	1.15	0.27
7	B	0.78	1.30	1.55
8	B	0.86	0.59	
9	B	1.20	0.51	0.20
10	A	0.34	0.20	0.77
11	A	2.18	2.11	0.99
12	A	0.29	0.44	0.64
13	B	0.91	0.60	1.09
14	A	1.12	0.58	0.27
15	A	0.41	0.11	0.58
16	A	1.47	0.30	3.87
17	A	0.45	2.04	0.72
18	B	0.80	1.62	0.88
19	B	0.51	4.57	
20	B	0.68		0.20
21	B	1.39	0.70	0.14
22	A	0.32	0.90	1.69
23	A	0.28	1.10	1.29
24	A	2.16	1.20	1.56
25	B	0.86	0.34	0.19
26	B	0.66	3.39	0.93
27	A	2.37	2.29	0.29
28	A	0.10	3.02	0.48
29	A	0.32	3.49	0.78
30	A	0.62	0.83	0.90
31	A	0.59	0.81	0.62
32	A	4.22	1.30	1.95
33	A	0.10	1.39	1.48
34	A	1.32	3.13	2.81
35	B	2.01	0.38	1.72
36	A	0.55	0.64	0.10
37	A	0.17	1.32	0.10

38	A	2.02	5.64	
39	A	0.10	0.62	1.22
40	A	1.06	0.91	0.72
41	A	1.21		0.66
42	A	0.72	1.12	1.52
43	A	0.46	0.38	0.66
44	B	1.22	0.74	0.68
45	B	0.79	0.92	1.03
46	B	1.42		0.81
47	B	2.66	1.06	0.60
48	B	0.46	1.62	0.77
49	B	0.74	1.39	1.01
50	B	0.10	0.66	1.16
	A = SIM+MCL			
	B= MCL			

APPENDIX K: GCF RAW CLINICAL DATA – VEGF-A

Patient	Group	VEGF -A baseline pg/30 sec sample	VEGF-A 2 week pg/30 sec sample	VEGF-A 12month pg/ 30 sec sample
1	B	0.75	7.52	1.63
2	B	1.74	5.86	1.75
3	A	2.38	4.91	3.35
4	B	1.67	0.93	2.41
5	B	1.51	4.93	2.20
6	B	8.52	6.35	3.99
7	B	6.85	5.70	4.32
8	B	2.63	8.04	
9	B	7.36	2.46	2.96
10	A	4.30	6.63	4.13
11	A	12.53	10.25	6.95
12	A	3.88	1.83	7.69
13	B	10.75	3.14	3.30
14	A	4.37	3.64	4.35
15	A	1.64	0.99	3.28
16	A	4.24	7.58	5.16
17	A	5.22	6.70	4.22
18	B	1.70	6.17	2.90
19	B	4.34	12.53	
20	B	5.22		3.88
21	B	4.87	7.20	2.12
22	A	3.23	10.56	4.80
23	A	9.38	15.79	8.28
24	A	11.82	4.63	5.77
25	B	5.35	12.23	0.83
26	B	5.52	9.31	3.07
27	A	12.09	20.93	7.30
28	A	2.63	5.83	1.06
29	A	0.69	2.51	0.97
30	A	3.67	2.06	1.06
31	A	0.90	9.25	1.35
32	A	5.99	9.17	5.77
33	A	2.72	4.47	1.70
34	A	0.46	2.69	2.69
35	B	9.61	3.88	6.40
36	A	11.69	3.33	2.45
37	A	4.90	4.13	3.78

38	A	4.22	5.09	
39	A	0.04	0.90	0.83
40	A	10.37	6.62	4.71
41	A	8.01		2.14
42	A	4.97	4.72	2.72
43	A	2.24	2.11	0.63
44	B	5.02	2.64	1.53
45	B	6.40	7.04	14.61
46	B	4.39		3.03
47	B	8.34	7.82	3.77
48	B	5.25	9.45	6.91
49	B	4.99	11.85	4.37
50	B	0.15	2.64	2.06
	A = SIM+MCL			
	B= MCL			

APPENDIX L: RT-PCR RAW CLINICAL DATA – FGF2

Patient	Group	FGF2 ΔΔCt Baseline	FGF2 Fold gene expression baseline	FGF2 ΔΔCt 2 week	FGF2 Fold gene expression 2 week
1	B	2.00	0.25		
2	B	1.63	0.32	-0.47	1.39
3	A	-0.51	1.43	-1.37	2.58
4	B			0.87	0.55
5	B	-0.44	1.35	1.52	0.35
6	B	1.46	0.36	1.36	0.39
7	B	-1.70	3.26	1.07	0.48
8	B	0.03	0.98	2.54	0.17
9	B	-1.23	2.34	-3.47	11.09
10	A	0.40	0.76	0.67	0.63
11	A	-0.40	1.32	-12.61	6253.97
12	A	-0.17	1.12	3.30	0.10
13	B	0.88	0.55	1.33	0.40
14	A	-0.90	1.86	2.05	0.24
15	A	-0.77	1.70	-0.85	1.80
16	A	0.02	0.99	-4.50	22.55
17	A	-0.12	1.09	-8.73	425.21
18	B	-2.47	5.53	0.56	0.68
19	B	-2.34	5.05	-5.05	33.19
20	B	0.13	0.91	2.08	0.24
21	B	-1.07	2.10	1.09	0.47
22	A	1.51	0.35	1.45	0.37
23	A	0.73	0.60	2.01	0.25
24	A	0.54	0.69	1.58	0.33
25	B	1.13	0.46	0.26	0.83
26	B	-0.94	1.92	1.57	0.34
27	A			2.07	0.24
28	A			1.30	0.41
29	A	0.19	0.88	0.50	0.71
30	A	1.52	0.35	-0.60	1.52
31	A	4.41	0.05	0.94	0.52
32	A	3.00	0.13	0.49	0.71
33	A	2.33	0.20	0.43	0.74
34	A	0.34	0.79	-0.86	1.82
35	B	2.80	0.14	-1.18	2.26
36	A	-0.87	1.82	-1.66	3.16
37	A	2.00	0.25	-1.42	2.68

38	A	0.53	0.69	-5.36	41.09
39	A	1.08	0.47	-2.83	7.12
40	A	1.49	0.36	2.99	0.13
41	A	-0.40	1.32		
42	A			2.06	0.24
43	A	-2.40	5.26	2.11	0.23
44	B	0.73	0.60		
45	B			0.38	0.77
46	B	-2.72	6.60	-0.86	1.81
47	B	-1.10	2.15	-0.95	1.93
48	B	-0.04	1.03	-1.55	2.92
49	B	2.89	0.13	-0.65	1.57
50	B	0.39	0.77	-0.47	1.38
	A = SIM+MCL				
	B= MCL				

APPENDIX M: RT-PCR RAW CLINICAL DATA – IL6

Patient	Group	IL-6 ΔΔCt baseline	IL-6 Fold gene expression baseline	IL-6 ΔΔCt 2-week	IL-6 Fold gene expression 2-week
1	B	3.52	0.09		
2	B	0.67	0.63	3.22	0.11
3	A	1.16	0.45		
4	B	1.26	0.42	4.52	0.04
5	B	0.27	0.83	6.08	0.01
6	B	-0.06	1.04	1.78	0.29
7	B	0.78	0.58	-2.32	4.98
8	B	-3.85	14.44	-0.70	1.63
9	B	-2.45	5.48		
10	A	-1.19	2.28	3.43	0.09
11	A	0.00	1.00	-7.04	131.21
12	A	1.73	0.30	3.32	0.10
13	B	-1.24	2.36	1.41	0.38
14	A	-0.44	1.36	-1.56	2.95
15	A			1.78	0.29
16	A	-0.71	1.63	-3.56	11.80
17	A	-0.13	1.09	-7.33	160.96
18	B	-3.95	15.46	-1.92	3.78
19	B	-2.59	6.02	-8.60	387.28
20	B	-1.43	2.69	0.54	0.69
21	B	-3.03	8.15	2.39	0.19
22	A	3.93	0.07	1.84	0.28
23	A	0.53	0.69	0.46	0.73
24	A	2.72	0.15	2.97	0.13
25	B	2.36	0.20	0.15	0.90
26	B	4.11	0.06	3.49	0.09
27	A			2.54	0.17
28	A			-1.20	2.30
29	A	2.80	0.14	0.36	0.78
30	A	4.56	0.04	-2.88	7.37
31	A	2.45	0.18	2.14	0.23
32	A	2.96	0.13	-0.82	1.77
33	A	0.23	0.85	2.89	0.14
34	A	1.78	0.29	-1.63	3.09
35	B	1.05	0.48	1.65	0.32
36	A	-4.36	20.53	2.45	0.18

37	A	3.18	0.11	-2.77	6.82
38	A	-0.01	1.01	-9.41	679.70
39	A	4.69	0.04	-6.35	81.50
40	A	2.02	0.25	5.49	0.02
41	A	-0.94	1.92		
42	A			-0.01	1.01
43	A	-1.61	3.05	-1.43	2.69
44	B	-0.06	1.04	2.40	0.19
45	B			-1.93	3.80
46	B	-4.88	29.35		
47	B	1.59	0.33	-5.54	46.67
48	B	1.61	0.33	-4.44	21.75
49	B	2.75	0.15	-2.12	4.35
50	B	3.56	0.08	-0.05	1.04
	A = SIM+MCL				
	B= MCL				

APPENDIX N: RT-PCR RAW CLINICAL DATA – IL10

Patient	Group	IL-10 ΔΔCt baseline	IL-10 Fold gene expression baseline	IL-10 ΔΔCt 2 week	IL-10 Fold gene expression 2 week
1	B	1.08	0.47		
2	B	0.20	0.87	2.91	0.13
3	A	2.82	0.14	0.18	0.88
4	B	1.42	0.37	1.82	0.28
5	B	1.22	0.43	1.18	0.44
6	B	1.64	0.32	-0.57	1.49
7	B	1.12	0.46	-2.12	4.36
8	B	0.18	0.88		
9	B	-1.42	2.68	-0.89	1.85
10	A	-0.62	1.54	-1.02	2.03
11	A	-1.56	2.95	-9.67	812.54
12	A	-2.55	5.85	0.49	0.71
13	B	-2.23	4.70		
14	A	0.38	0.77	1.59	0.33
15	A	-3.64	12.48	0.47	0.72
16	A	-2.89	7.42	-3.27	9.68
17	A	1.22	0.43	-7.86	232.19
18	B	-2.74	6.67	1.44	0.37
19	B	-4.64	24.88	-4.49	22.52
20	B	0.12	0.92	0.75	0.59
21	B	-3.57	11.87	7.21	0.01
22	A	1.84	0.28	3.33	0.10
23	A	1.38	0.38	1.19	0.44
24	A	1.99	0.25	0.62	0.65
25	B	0.68	0.62	-1.49	2.81
26	B	4.26	0.05	0.58	0.67
27	A			-1.24	2.36
28	A			-1.15	2.21
29	A	0.21	0.87	2.01	0.25
30	A	3.34	0.10	-1.08	2.11
31	A	1.73	0.30	0.28	0.83
32	A	3.51	0.09	1.41	0.38
33	A	0.99	0.50	-2.94	7.69
34	A	2.24	0.21	-1.84	3.59
35	B	2.49	0.18	-1.88	3.67
36	A	-2.80	6.97	1.02	0.49
37	A	1.65	0.32	-1.63	3.09

38	A	-0.03	1.02	-4.11	17.23
39	A	5.99	0.02	-4.46	21.97
40	A	-1.15	2.21	0.84	0.56
41	A	-0.29	1.22		
42	A			0.71	0.61
43	A	-1.71	3.28	-1.26	2.39
44	B	-0.23	1.17	-0.05	1.03
45	B			-1.91	3.76
46	B	-2.09	4.27		
47	B	0.39	0.77	-1.56	2.94
48	B	2.30	0.20	0.16	0.90
49	B	-1.48	2.79	-0.47	1.39
50	B	1.31	0.40	-0.62	1.53
	A = SIM+MCL				
	B= MCL				

APPENDIX O: RT-PCR RAW CLINICAL DATA – IL17

Patient	Group	IL-17 ΔΔCt baseline	IL-17 Fold gene expression baseline	IL-17 ΔΔCt 2 week	IL-17 Fold gene expression 2 week
1	B	3.78	0.07		
2	B	1.86	0.28	1.26	0.42
3	A	2.07	0.24	3.27	0.10
4	B	-0.07	1.05	-1.64	3.12
5	B	1.29	0.41	6.12	0.01
6	B	-2.73	6.65	4.79	0.04
7	B			1.23	0.43
8	B			-2.61	6.12
9	B	-1.29	2.45	-4.55	23.44
10	A	0.30	0.81	1.72	0.30
11	A			-10.25	1213.89
12	A	-1.09	2.13	8.23	
13	B	-2.13	4.38	-1.27	2.41
14	A	-1.09	2.13	2.22	0.21
15	A	-1.84	3.58	-0.74	1.67
16	A	-1.46	2.75	-5.54	46.55
17	A	-1.69	3.22	-9.85	925.93
18	B	-3.29	9.78	5.13	0.03
19	B	-3.04	8.21	-3.88	14.76
20	B	0.46	0.73		
21	B	-4.01	16.14	4.58	0.04
22	A	4.07	0.06	3.31	0.10
23	A	-0.29	1.22	1.82	0.28
24	A			1.95	0.26
25	B	4.13	0.06	-1.20	2.29
26	B			1.15	0.45
27	A			1.06	0.48
28	A			2.82	0.14
29	A	2.52	0.17	2.60	0.16
30	A	2.85	0.14	0.11	0.93
31	A	2.89	0.13	3.68	0.08
32	A	1.42	0.37	1.57	0.34
33	A	0.61	0.65	-0.56	1.47
34	A	5.39	0.02	0.89	0.54
35	B	3.32	0.10	0.03	0.98
36	A	0.39	0.76	0.13	0.91
37	A	1.46	0.36	1.85	0.28

38	A	3.55	0.09	-3.87	14.64
39	A	3.45	0.09	-1.84	3.58
40	A	-0.27	1.21	0.69	0.62
41	A	0.01	0.99		
42	A			-0.06	1.04
43	A	3.15	0.11	-1.56	2.94
44	B	0.94	0.52	1.30	0.41
45	B			-2.05	4.13
46	B	-5.19	36.55		
47	B	1.91	0.27	2.42	0.19
48	B	1.51	0.35	2.16	0.22
49	B	0.40	0.76		
50	B	2.16	0.22	0.88	0.54
	A = SIM+MCL				
	B= MCL				

APPENDIX P: RT-PCR RAW CLINICAL DATA – IGF1

Patient	Group	IGF1 ΔΔCt baseline	IFG1 Fold gene expression baseline	IGF1 ΔΔCt 2 week	IGF1 Fold gene expression 2 week
1	B	0.79	0.58		
2	B	1.07	0.48	0.12	0.92
3	A	0.68	0.62		
4	B	1.92	0.26	1.86	0.28
5	B	0.08	0.94	-1.10	2.14
6	B	2.25	0.21	-0.93	1.90
7	B	1.14	0.45	0.70	0.62
8	B	-0.62	1.54	1.15	0.45
9	B	-0.67	1.59	-3.78	13.74
10	A	1.00	0.50	0.98	0.51
11	A	-1.12	2.18	-10.52	1472.95
12	A	1.47	0.36	3.78	0.07
13	B	0.00	1.00	1.15	0.45
14	A	0.18	0.88	1.16	0.45
15	A			0.83	0.56
16	A	-2.14	4.41	-3.71	13.07
17	A	-0.07	1.05	-7.90	239.12
18	B	-2.53	5.79	3.14	0.11
19	B	-3.16	8.93	-1.00	2.00
20	B	0.24	0.84	2.45	0.18
21	B	-2.13	4.37	-0.26	1.19
22	A	2.70	0.15	0.46	0.73
23	A	-0.18	1.14	3.07	0.12
24	A	2.28	0.21	1.37	0.39
25	B	1.19	0.44	0.23	0.85
26	B	2.25	0.21	0.56	0.68
27	A			1.77	0.29
28	A			0.46	0.73
29	A	1.91	0.27	1.34	0.39
30	A	1.88	0.27	-1.16	2.23
31	A	0.24	0.85	0.04	0.97
32	A	1.25	0.42	0.74	0.60
33	A	1.31	0.40	-0.37	1.29
34	A	1.42	0.37	-2.65	6.29
35	B	1.98	0.25	-1.76	3.38
36	A	-0.06	1.04	-2.34	5.08

37	A	1.50	0.35	-0.77	1.71
38	A	-0.03	1.02	-1.41	2.65
39	A	0.31	0.81	-2.81	7.01
40	A	0.55	0.68	0.41	0.75
41	A	-1.23	2.34		
42	A			2.30	0.20
43	A	-3.17	9.03	1.80	0.29
44	B	0.41	0.75	0.00	1.00
45	B			-3.08	8.43
46	B	-2.95	7.73		
47	B	-1.13	2.19		
48	B	-0.02	1.02	0.12	0.92
49	B	0.25	0.84	1.04	0.49
50	B	-0.36	1.28	-0.63	1.55
	A = SIM+MCL				
	B= MCL				

APPENDIX Q: RT-PCR RAW CLINICAL DATA – RANKL

Patient	Group	RANKL ΔΔCt Baseline	RANKL Fold gene expression Baseline	RANKL ΔΔCt 2 week	RANKL Fold gene expression 2 week
1	B	0.44	0.74		
2	B	0.64	0.64	-1.84	3.57
3	A	2.46	0.18	-0.09	1.06
4	B	1.32	0.40	0.32	0.80
5	B	0.33	0.79	-0.34	1.27
6	B	1.86	0.28	-1.13	2.19
7	B	1.72	0.30	0.86	0.55
8	B	1.58	0.33	0.35	0.78
9	B			-3.44	10.82
10	A	0.58	0.67	-0.16	1.12
11	A	1.57	0.34	-9.39	673.19
12	A	-1.33	2.51	0.84	0.56
13	B	-0.88	1.84	4.35	0.05
14	A	0.17	0.89	0.69	0.62
15	A	-0.88	1.84	3.47	0.09
16	A	-0.24	1.18	-1.02	2.02
17	A	0.41	0.75	-4.93	30.52
18	B	-1.13	2.18	1.42	0.37
19	B	-2.42	5.37	-1.26	2.39
20	B	-0.92	1.89	1.26	0.42
21	B	-0.57	1.48	0.03	0.98
22	A	0.46	0.73	-0.76	1.70
23	A	1.18	0.44	1.91	0.27
24	A	0.81	0.57	1.19	0.44
25	B	-0.39	1.31	-0.52	1.44
26	B	0.39	0.76	0.08	0.94
27	A			0.79	0.58
28	A			0.93	0.53
29	A	1.32	0.40	-0.47	1.39
30	A	1.24	0.42	0.77	0.59
31	A	2.14	0.23	-1.29	2.44
32	A	2.77	0.15	2.34	0.20
33	A	2.30	0.20	-2.19	4.56
34	A	1.08	0.47	-0.52	1.44
35	B	1.87	0.27	-1.18	2.26
36	A	-0.51	1.43	-2.15	4.44
37	A	1.83	0.28	-0.64	1.56

38	A	1.03	0.49	-0.92	1.89
39	A	-0.79	1.73	-2.91	7.53
40	A	1.51	0.35	0.28	0.82
41	A	0.56	0.68		
42	A			0.51	0.70
43	A	-1.67	3.18	-0.30	1.23
44	B	-0.08	1.06	-0.32	1.24
45	B			-1.25	2.38
46	B	-0.45	1.36		
47	B	0.31	0.80	0.60	0.66
48	B	-0.77	1.70	1.06	0.48
49	B	-1.40	2.64	0.75	0.59
50	B	-1.47	2.77	0.18	0.88
	A = SIM+MCL				
	B= MCL				

APPENDIX R: 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

IRB Approved
Valid until 03/21/2020

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

Institutional Review Board (IRB)

What Do I Need To Know Before Being In A Research Study?

You have been invited to be in a **research study**. Research studies are also called "clinical trials" or "protocols." **Research** is an organized plan designed to get new knowledge about a disease or the normal function of the body. The people who are in the research are called **research subjects**. The **investigator** is the person who is running the research study. You will get information from the investigator and the research team, and then you will be asked to give your **consent** to be in the research.

This sheet will help you think of questions to ask the investigator or his/her staff. You should know all these answers before you decide about being in the research.

What is the **purpose** of the research? Why is the investigator doing the research?

What are the **risks** of the research? What bad things could happen?

What are the possible **benefits** of the research? How might this help me?

How is this research different than the care or treatment I would get if I wasn't in the research? Are there other treatments I could get?

Does **everyone** in this research study get the same treatment?

Will being in the research **cost** me anything extra?

Do I have to be in this research study? Will the doctor still take care of me if I say **no**?

Can I **stop** being in the research once I've started? How?

Who will look at my **records**?

How do I reach the investigator if I have more **questions**?

Who do I call if I have questions about being a **research subject**?

Make sure all your questions are answered before you decide whether or not to be in this research.

Academic Research & Services Building 3000 / 987830 Nebraska Medical Center / Omaha NE 68198-7830
402-559-6463 / FAX 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

Institutional Review Board (IRB)

THE RIGHTS OF RESEARCH SUBJECTS
AS A RESEARCH SUBJECT YOU HAVE THE RIGHT □

□**to be told everything you need to know about the research before you are asked to decide whether or not to take part in the research study.** The research will be explained to you in a way that assures you understand enough to decide whether or not to take part.

□**to freely decide whether or not to take part in the research.**

□**to decide not to be in the research, or to stop participating in the research at any time.** This will not affect your medical care or your relationship with the investigator or the Nebraska Medical Center. Your doctor will still take care of you.

□**to ask questions about the research at any time.** The investigator will answer your questions honestly and completely.

□**to know that your safety and welfare will always come first.** The investigator will display the highest possible degree of skill and care throughout this research. Any risks or discomforts will be minimized as much as possible.

□**to privacy and confidentiality.** The investigator will treat information about you carefully, and will respect your privacy.

... to keep all the legal rights you have now. You are not giving up any of your legal rights by taking part in this research study.

□**to be treated with dignity and respect at all times**

The Institutional Review Board is responsible for assuring that your rights and welfare are protected. If you have any questions about your rights, contact the Institutional Review Board at (402) 559-6463.

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