Study Protocol

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

NCT: 03452891

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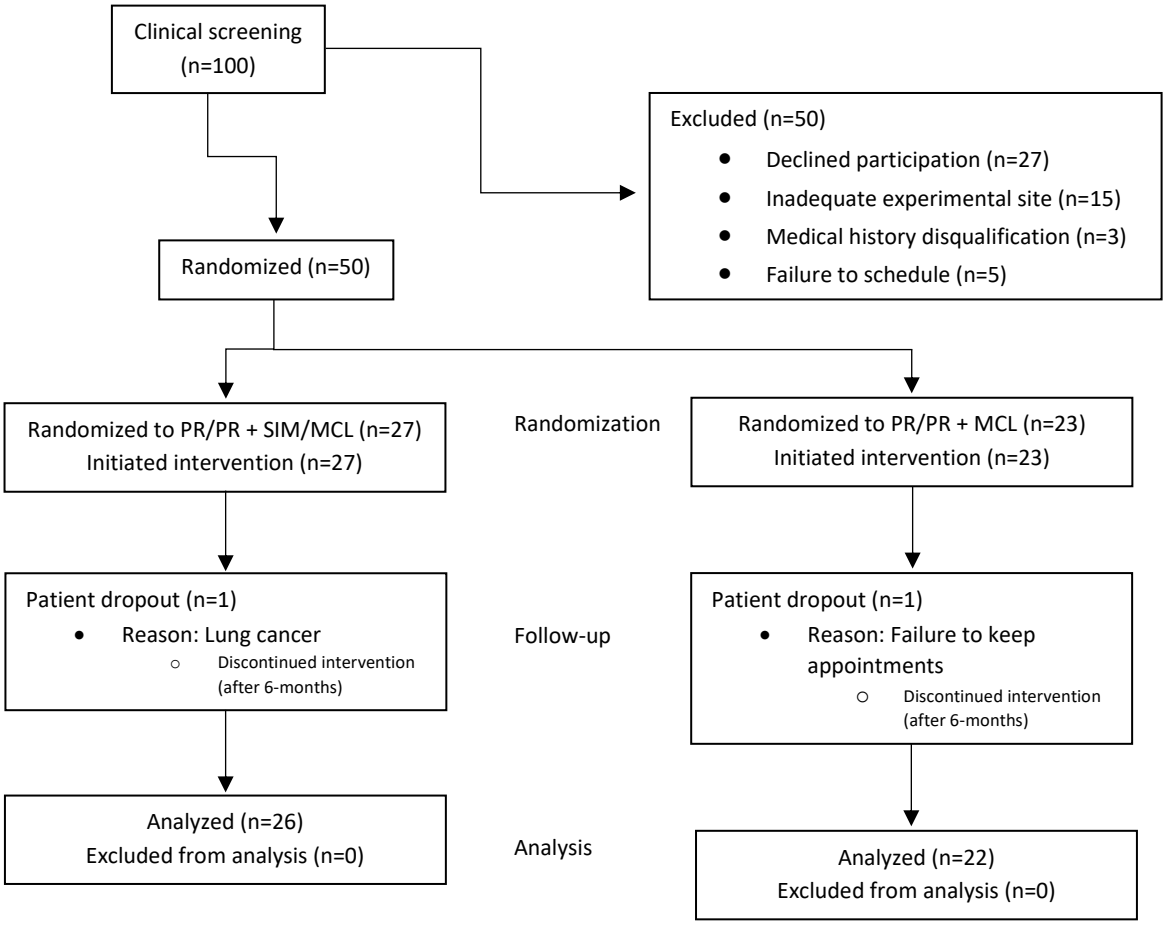
Patient Population

This one year, randomized, and double-masked clinical trial was conducted from March 2019-December 2020. Fifty patients, who were receiving periodontal maintenance therapy at the University of Nebraska Medical Center (UNMC) College of Dentistry were screened and identified by faculty and investigators involved in this study (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


Clinical screening
(n=100)
Excluded (n=50)
Declined participation (n=27)
Inadequate experimental site (n=15)
Medical history disqualification (n=3)
Failure to schedule (n=5)
Randomized (n=50)
Randomized to PR/PR + SIM/MCL (n=27)
Initiated intervention (n=27)
Randomization
Randomized to PR/PR + MCL (n=23)
Initiated intervention (n=23)
Patient dropout (n=1)
Reason: Lung cancer
Discontinued intervention (after 6-months)
Follow-up
Patient dropout (n=1)
Reason: Failure to keep appointments
Discontinued intervention (after 6-months)
Analyzed (n=26)
Excluded from analysis (n=0)
Analysis
Analyzed (n=22)
Excluded from analysis (n=0)

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, experimental site (6-9 mm interproximal pocket with no vertical bony ≥ 1.5 mm, and history of bleeding on probing) was isolated with cotton rolls and gently dried with gauze. Supragingival plaque was removed from the test 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 vial of 1.5 mL RNA*later* solution (ThermoFisher Scientific, Waltham, MA) (Figure 3) and frozen at -20°C until further analysis. Scaling and root planning 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 unmasked 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 papilla 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

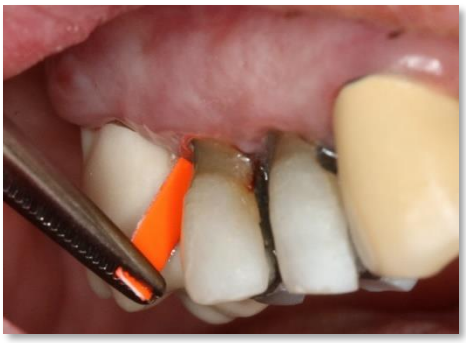


Figure 3: Baseline Interproximal tissue

Figure 4: Perioscope use

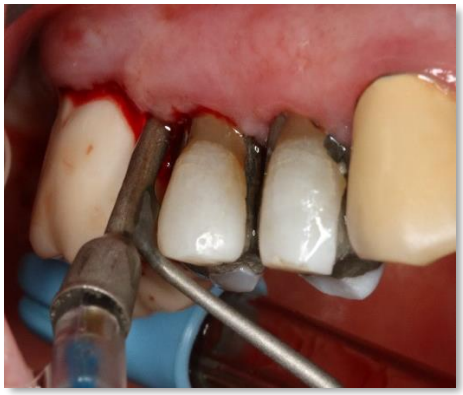


Figure 5: Mixing SIM & MCL

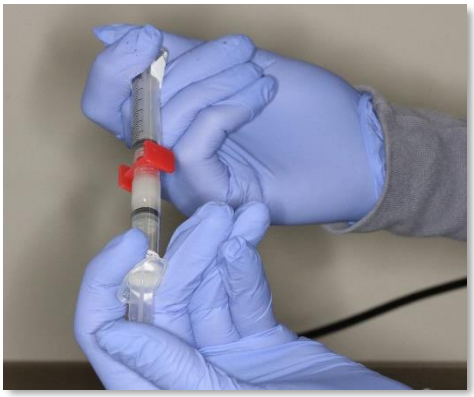


Figure 6: Placement of SIM

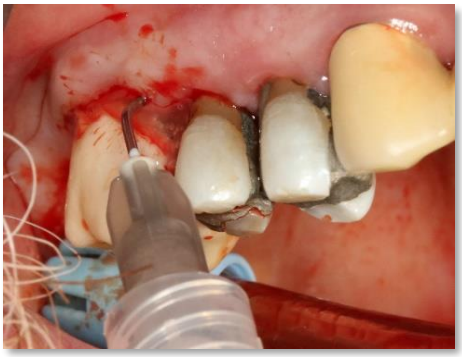
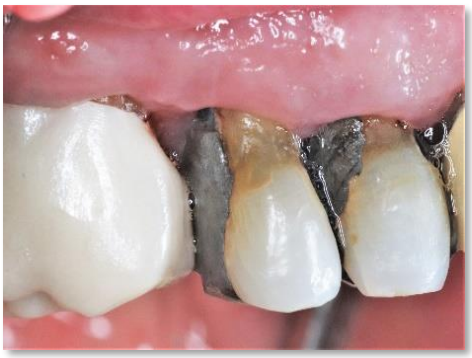


Figure 7: 12-months post-therapy



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.

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 value, 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).