Title

Effect of subgingival irrigation with 0.05% sodium hypochlorite as adjunct to scaling and root planing on subgingival microbiota and gingival inflammation in moderate to severe chronic periodontitis patients

Author

Tamhane, Neha

Publication Date

2019

Peer reviewed|Thesis/dissertation

Effect of subgingival irrigation with 0.05% sodium hypochlorite as adjunct to scaling and root planing on subgingival microbiota and gingival inflammation in moderate to severe chronic periodontitis patients

by

Neha Tamhane

THESIS

Submitted in partial satisfaction of the requirements for degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

DocuSigned by:
Yvonne Kapila
CE032F3A4E984CF...

Yvonne Kapila

Chair

DocuSigned by:
PINELOPI XENOUDI
DocuSigned by:
MIKE A SABETI
94AE03CFE96647C...

PINELOPI XENOUDI

MIKE A SABETI

Committee Members

Acknowledgements

Division of Periodontology and Department of Orofacial Sciences

at University of California, San Francisco

uBiome, Inc.

Dr. Allan Radaic, Orofacial Sciences

Dr. Pachiyappan Kamarajan, Orofacial Sciences

Committee in Charge:

Dr. Yvonne Kapila, Dr. Mike Sabeti, and Dr. Pinelopi Xenoudi

Thank you for your continued support and guidance

To my wonderful family, my fiancé, my co-residents, staff, and faculty, thank you for your love and support!

Abstract

Effect of subgingival irrigation with 0.05% sodium hypochlorite as adjunct to scaling and root planing on subgingival microbiota and gingival inflammation in moderate to severe chronic periodontitis patients

Neha Tamhane

Objective

This study aims to evaluate the use of sodium hypochlorite (NaOCl) irrigation as adjunctive therapy to scaling and root planing for periodontal disease. The study focuses on the potential effects of NaOCl on the sub-gingival microbiological composition and clinical periodontal parameters.

Methods

This study is a single site, randomized clinical trial with parallel arms conducted on subjects who have been diagnosed with moderate to severe periodontitis based on clinical examination. Clinical periodontal parameters, including plaque index, bleeding on probing, probing pocket depth, and clinical attachment loss, were measured at baseline and at 4-6 weeks. Subjects were randomly treated with either scaling and root planing with water irrigation or scaling and root planing with 0.05% NaOCl irrigation. Subgingival microbial samples were collected from two sites per subject at baseline and at final evaluation at 4-6 weeks. The microbial samples were analyzed using 16S rRNA gene sequencing.

Results

A total of eight subjects completed the study. There was a decrease in pocket depth, clinical attachment loss, bleeding on probing, and plaque in both groups after 4-6 weeks, but the

differences between the groups were not statistically significant. The analysis of the microbiome revealed some trends, although they were not statistically significant. The diversity increased in the experimental group. There was a decrease in the percent composition of *Porphyromonas* at the final evaluation in both groups. The experimental group had an increase in percent composition of *Haemophilus*, *Streptococci*, and *Veillonella* at the final evaluation.

Conclusion

Although this was a small study that showed no difference between irrigation with 0.05% NaOCl and water, the effect of scaling and root planing on the clinical parameters of periodontal disease is observed. The control and experimental groups improved across all clinical parameters at the final evaluation. This study provided further insight into the antimicrobial effects of NaOCl. The decrease in percent composition of the *Porphyromonas* genera, which contains a key-stone periodontal pathogen, may be considered favorable. The experimental group had increases in percent composition of several genera that are generally considered to be commensals. With further research, NaOCl has the potential to be an affordable and widely accessible treatment modality for periodontitis patients worldwide.

Table of Contents **Page**

List of Figures **Page**

List of Tables **Page**

I. Introduction

I. Introduction

Periodontitis is a widespread, multifactorial disease that affects almost half of the adult population in the United States and mediates a high global burden disease, and it has significant socioeconomic implications. [1,2] According to the 2010 Global Burden of Disease Study, severe periodontitis is the 6th most prevalent disease in the world, affecting 743 million people around the world.[3–5] Periodontitis can be a debilitating disease marked by tooth loss, lack of masticatory function, inadequate nutritional status, and poor quality of life that leads to disability.[6]

Periodontitis in humans has been documented for centuries with reports of loose teeth and bleeding gums, but the nomenclature, causes, and treatments have changed and evolved.[7] The current school of thought is that periodontitis is a dysbiosis between the host immune response and the oral microbiota, influenced by genetic and environmental factors.[8] Bacteria form a biofilm on the tooth and gingival tissues. If a biofilm is left undisrupted, it may favor the rise of more pathogenic microbes that trigger a host response, including gingival inflammation.[8] In a susceptible host, a disproportionate host response characterized by excessive cytokines, prostaglandins, and matrix metalloproteinases contributes to bone resorption and connective tissue breakdown. The dentition is compromised as pockets deepen around the teeth and there is loss of clinical attachment and alveolar bone.[8,9] If this continues, the teeth become mobile and may be lost. The host response to the microbiota is influenced by genetics, but can be modified by other factors such as smoking and poorly controlled diabetes.[8]

Understanding the etiology of periodontitis is imperative before determining the course of treatment. Bacterial plaque and its byproducts are one of the primary etiologies of periodontitis.

Historically, there were two schools of thought regarding the etiology of periodontal diseases. According to the specific theory, periodontitis is caused by the presence of specific pathogens and treatment should focus on the elimination of those pathogens.[10] Several pathogens known as the red complex are frequently associated with periodontitis. The complex consists of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*.[11] Studies have shown a greater presence of these pathogens in patients with chronic periodontitis than in periodontally healthy people. *P. gingivalis,* a Gram-negative anaerobe bacterium, is considered a key-stone pathogen in developing periodontitis due to its virulence.[12] Conversely, the non-specific theory states that periodontal diseases are caused by the proliferation of indigenous oral bacteria and their combined virulence factors.[13] There is a state of dynamic equilibrium maintained between the host and their oral microbiota. The equilibrium can be disrupted by an increasing mass of the oral microbiota and an increased effect of virulence factors of the specific bacteria present.[14] In this model, treatment is focused on elimination of the bacterial plaque, as its increasing biomass is thought to cause the disease.[13]

Altering the composition of the subgingival biofilm and attempting to remove the microbial mass to reduce inflammation is the primary purpose of conventional periodontal therapy. Scaling and root planing (SRP) is the gold standard of non-surgical therapy for periodontitis.[15–17] The objective of SRP is twofold. Scaling is the removal of biofilm, in the form of plaque and calculus, from a tooth surface.[18] Root planing is the removal of rough cementum or dentin that is contaminated with microbes.[18] According to the American Dental Association's Council on Scientific Affairs, patients with chronic periodontitis benefit moderately from SRP. The clinical practice guidelines are in favor of SRP as the initial nonsurgical treatment for chronic

periodontitis.[19] Studies have shown that SRP is effective in reducing the bacterial load and the number of specific periodontal pathogens.[20] It is important to reduce the burden of specific pathogens such as *P. gingivalis* because there is a positive correlation between pocket depth reduction and decrease in *P. gingivalis*.[21] SRP has been shown to reduce gingival inflammation in patients with periodontitis.[22] In spite of its effectiveness in reducing gingival inflammation and improving periodontal health, there are limitations to the procedure. Multiple studies have shown that complete or near complete removal of plaque and calculus in periodontal pockets deeper than 4-5 mm is not possible.[23–25] The effectiveness of biofilm removal during SRP is also affected by the anatomy of the teeth, root proximity, and inaccessible furcation entrances.[26] Several periodontal pathogens are difficult to eliminate with SRP alone and may need surgical intervention.[27] Periodontal pockets greater than 4.2mm benefit from clinical attachment gain following surgical treatment, however periodontal maintenance therapy at 3-month intervals is necessary to maintain the results.[28] Based on the proceedings of the 2017 World Workshop, a patient diagnosed with periodontitis retains that diagnosis for a lifetime because there remains a risk of disease recurrence even with successful treatment.[29]

Various antimicrobials have been used locally and systemically as monotherapy and adjunctive therapy in patients with periodontitis. NaOCl is considered a common and effective agent in endodontic therapy because of its antimicrobial property and ability to dissolve pulpal matter inside the root canal system.[30] It has not been as widely explored as an antimicrobial in patients with periodontitis as it has in endodontic treatment. The mechanism of action of this solution is through oxidation of proteins, nucleotides and lipids. Hypochlorite ion is produced naturally by human neutrophils and macrophages in minute quantities and plays an important role in the

host's innate immune response.[31] NaOCl solutions have antimicrobial activity against *E. Faecalis* biofilms inside of dentinal tubules.[32] It has also been shown to eliminate *P. gingivalis* within 15 seconds in vitro at all concentrations from 0.5% to 5.25%.[33] NaOCl has dose-dependent effects, and thus higher concentrations are more effective at killing bacteria. At higher concentrations, NaOCl can remove organic tissue, making it a highly effective agent in endodontic therapy.[34] NaOCl irrigation in root canal preparations removes pulpal tissue completely at concentrations of 1%, 2.5%, and 5.25%. At a concentration of 0.5%, some remnants of pulpal tissue are still detectable.[35] In NaOCl solutions with concentrations of 1% to 5.8%, the amount of pulpal tissue dissolution increased almost linearly with increasing concentrations.[36] Generally, the effective concentration of NaOCl in endodontics ranges from 2.6% – 5.25%.[35]

The use of NaOCl is rare in treating other infections beside those of endodontic origin. The application of NaOCl in periodontal treatment has not been widely explored. One study by De Nardo et al. evaluated the effect of a 0.05% NaOCl rinse in an experimental gingivitis model compared to a water rinse. The NaOCl group showed 48% reduction in dental plaque along with a reduction in gingival inflammation compared to the water rinse group.[37] A 0.1% concentration of NaOCl has been recommended as a mild antiseptic mouth rinse by the American Dental Association Council on Dental Therapeutics.[38] Another recent study by Galván et al. evaluated the use of a 0.25% NaOCl oral rinse twice-a-week in the absence of scaling and root planing. Significant reduction in bleeding on probing was observed, even in deep unscaled pockets.[39] An in vitro model examined the antimicrobial activity of 0.95% NaOCl gel compared to 0.1% chlorhexidine digluconate solution on biofilms containing periodontal pathogens. Compared to

chlorhexidine, the NaOCl gel inhibited more growth of Gram-negative bacteria compared to chlorhexidine on newly formed biofilms and had a greater bacteriocidal effect on 4-day old biofilms.[40] A randomized clinical trial by Bizzarro et al. evaluated the use of professionally administered 0.5% NaOCl irrigation compared to saline irrigation during SRP with and without systemic antibiotics (amoxicillin and metronidazole). The group found no lasting effect of a single session of NaOCl irrigation after 12 months, which suggests that the effects are short-lived. However, 22% of their subjects reported adverse reactions to the systemic antibiotics and no adverse reactions to the irrigation.[41]

Local delivery of antimicrobials compared to systemic drug delivery achieves higher concentrations in subgingival sites.[42] Local delivery can be performed by patients as part of oral hygiene at home or by the dental provider. Self-application by the patient allows for more frequent use of the antimicrobial. However, application by patients can be limited by lack of manual dexterity and difficulty in understanding dental anatomy.[43] Application of a local antimicrobial agent in a professional setting also ameliorates the issue of patient compliance with systemic antibiotic therapy.[43] Pitcher et al. compared the penetration of mouthwashes to irrigation right at the entrance of the pocket. They found that mouthwash only penetrated about 0.2mm into the pocket, while irrigation without entering the pocket penetrated on average 1.8mm.[44] Irrigation with an erythrosin dye solution was found to penetrate 90% into pockets up to 6mm when the syringe tip was placed 1-2 mm subgingivally and irrigated 5 seconds compared to 21% penetration with oral rinsing. In pockets 7mm or greater, the penetration was 64%.[45] Eakle et al. found a 44-71% penetration with the WaterPik oral irrigator using erythrosin dye.[46] In a study by Soh et al., subjects instructed to self-irrigate daily with chlorhexidine had

significantly lower pocket depths and plaque at 28 days after SRP compared to the control subjects.[47]

NaOCl usage, if extended to periodontitis patients, must be at a lower concentration to avoid tissue dissolution. It is important to note that NaOCl is a potent antimicrobial, which carries the risk of toxicity. At high concentrations, its cytotoxicity and tissue toxicity are well-documented.[30] When NaOCl contacts the tissue, proteins are dissolved and other proteins, nitrogen, formaldehyde, and acetaldehyde are formed quickly. Necrotic tissue is also removed.[48] Reports of hypersensitivity reactions to NaOCl are rare, but they have been documented in case reports. A case report by Kaufman et al. describes a patient who was diagnosed with hypersensitivity to household bleach with a skin patch test. The patient received endodontic treatment with another irrigating agent with no complications.[49] A second case report by Caliskan et al. reported on a patient who received 1% NaOCl irrigation during endodontic treatment and developed difficulty breathing, swelling, and pain. A skin scratch test revealed an allergic reaction to NaOCl.[50]

The goal of this study was to test the short-term effect of a low concentration of NaOCl on periodontitis. Based on the previous studies that used NaOCl in periodontitis, we selected a low concentration of 0.05% NaOCl. Irrigation by a dental provider is the modality of choice, since it addresses the issue of patient compliance and patient ability to dilute the solution accurately. The effect of the irrigation on the microbiota may be transient so we evaluated changes 4-6 weeks after irrigation.

We hypothesized that subgingival irrigation with NaOCl as an adjunct to SRP would enhance clinical outcomes as a result of a greater suppression and alteration of the subgingival microbiota than SRP alone. We tested this hypothesis using a randomized clinical trial.

II. Materials and Methods

II. Materials and Methods

This study was approved by the UCSF Institutional Review Board on 06/06/2018 and assigned IRB number 18-24359.

This study was a single site, single blinded, randomized clinical trial. Subjects were randomly assigned to one of two treatment groups: SRP with NaOCl irrigation and SRP with water irrigation. Clinical examinations that included periodontal measurements were conducted at baseline and at final evaluation at 4-6 weeks. Irrigation was provided once at the initial visit immediately after SRP. Subgingival microbial samples were collected at baseline and at the final evaluation.

Subject Recruitment and Characteristics

Subjects were recruited prospectively from the new patient pool in the Periodontology Clinic at the University of California, San Francisco. Subjects were patients of the resident providers. They had completed their initial examination and were identified based on their need for SRP. All subjects were diagnosed with moderate to severe chronic periodontitis.[51] Final selection of subjects was based on the inclusion and exclusion criteria listed below. Patient records, including periodontal examination charts and medical history, were reviewed to determine subject eligibility.

Table 2.1 Study Design and Timeline

Appointments	**Control Group** **SRP plus Water** **Sample size = 4 subjects**	**Test Group** **SRP plus NaOCL** **Sample size = 4 subjects**
Pre-Study appointment	Baseline evaluation comprehensive periodontal exam with clinical measures	Baseline evaluation comprehensive periodontal exam with clinical measures
Study visit #1 Week 0	Informed Consent Obtained Microbial sampling SRP Oral hygiene instructions Subgingival irrigation with water	Informed Consent Obtained Microbial sampling SRP Oral hygiene instructions Subgingival irrigation with NaOCl
Study visit #2 Week 4-6	Final evaluation comprehensive periodontal exam with clinical measures Microbial Sampling	Final evaluation comprehensive periodontal exam with clinical measures Microbial Sampling

Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Patients had to be 18 years and older

2. Patients had to be systemically healthy or with mild systemic disease based on the American Society of Anesthesiologists (ASA) physical status classification system. Patients were classified as ASA I and II only.[52]

3. Patients had to have an established diagnosis of moderate to severe chronic periodontitis.[51] Patients had to have at least 3-4 mm of attachment loss along with a minimum of 5 mm of probing depths on at least 2 non-adjacent teeth in a single quadrant.[53]

4. Patients had to be able to sign their own consent form and possess decision-making ability.

Exclusion Criteria:

1. Presence of any disease or medication that alters the immune system or interferes with healing ability

2. Heavy tobacco use (greater than 10 cigarettes per day)

3. External or internal tooth resorption

4. Necrotic or endodontically involved teeth

5. Pregnant or nursing because hormonal factors could influence the condition

6. Patients who are known to be sensitive or report allergy to NaOCl

7. Any other conditions that interfere with periodontal evaluation

8. Systemic antibiotic use in the past 6 months

9. Current oral or dental pathology

Enrollment

Prospective subjects were patients of the clinic whose treatment included SRP. If the patients were interested in participating in the study, the findings from their periodontal evaluation were reviewed, including medical history and periodontal parameters, to determine eligibility. Subjects who met the study inclusion criteria and consented to participate were enrolled in the study.

Randomization Procedure

This was a single-site randomized clinical trial in which participants were randomized to one of two arms: i) SRP with NaOCL irrigation or ii) SRP with water irrigation. At enrollment, the subjects were assigned to either Group A (NaOCl) or Group B (water); enrollment continued in an alternating pattern.

Duration of the study

The duration of each subject's participation was 4-6 weeks.

Study Plan

The clinical examinations were conducted by the patients' assigned periodontal providers prior to enrollment in the study. In this study, the providers were all residents of the UCSF Periodontology Clinic. The providers informed their patients of the ongoing study. One examiner (N.T.) explained the study and answered all questions for the patients, if they expressed interest in participating. After obtaining informed consent enrollment, the microbial sampling was performed by two examiners (Y.K. and M.S.) at the following appointment, prior to SRP. SRP was also conducted by the patients' providers as part of the patients' routine periodontal treatment. The irrigation was performed by one examiner (N.T.) immediately after the SRP procedure. All subjects were given oral hygiene instructions at the time of their SRP appointment by their provider. Subjects were asked to avoid the use of any mouthwash or mouth rinse other than water for the duration of their participation in the study to avoid confounding effects. At the final evaluation appointment 4-6 weeks after the SRP, study examiners (Y.K. and M.S.)

performed the microbial sampling. The patients' providers performed the same clinical examination after the microbial sampling was completed.

Blinding

A single blind study design was adopted. The periodontal providers performing the exams and conducting the SRP were blinded to the type of irrigation received by their patients. However, the subjects could not be blinded due to the difference in taste and odor of the two irrigation modalities.

Clinical Evaluations

At baseline and final visits, a complete periodontal examination was performed by the subject's periodontal provider. Several measurements were recorded as part of the comprehensive periodontal evaluation, including pocket depth (PD); bleeding on probing (BOP); plaque index (PI); and the clinical attachment loss (CAL).

Pocket Depth

Pocket depth is the distance in millimeters gingival margin to the bottom of the gingival crevice. Each measurement is obtained from 6 sites on each tooth. PD is an important measurement because it influences the difficulty of subgingival plaque and calculus removal. PD is an important factor in treatment planning because shallow and deep pockets necessitate different treatment. SRP on pockets less than 2.9 mm can cause additional loss of attachment.[28]

Bleeding on Probing

Bleeding on probing (BOP) is a commonly used method of diagnosing gingival inflammation and has some limited predictability for future attachment loss.[54] However, the absence of BOP is highly accurate predictor of future attachment loss. A study by Lang et al. showed that 98.5% of sites that did not have BOP did not have future attachment loss.[54] BOP is measured dichotomously based on its presence or lack at each of six sites on a tooth as the pockets are probed.[55] If bleeding is present at a site, the corresponding site on the periodontal chart is marked with a 1. If there is no bleeding, the site is marked with a 0. This is repeated for all six sites per tooth in the selected quadrant. The quantity and duration of BOP is not recorded as part the clinical evaluation nor the study.

Plaque Index

Plaque index (PI) is measured dichotomously based on its presence or lack at each of six sites on a tooth. If plaque is present at a site, the corresponding site on the periodontal chart is marked with a 1. If there is no plaque, the site is marked with a 0.

Clinical Attachment Loss

The clinical attachment loss (CAL) is the distance in millimeters from the cementoenamel junction of the tooth to the bottom of the gingival crevice. The number can be calculated by adding probing depth of the pocket to distance of gingival margin from the cementoenamel junction. Each measurement is obtained from 6 sites on each tooth. The CAL is important in diagnosing periodontitis because it indicates the extent of periodontal attachment loss around a tooth.

Site selection

One quadrant was selected per subject. The quadrant had to include a minimum of two sites with probing depths of 5 mm of greater. Two sites with probing depths of 5 mm or greater were used for microbial sampling. All sites within the quadrant with probing depths of 5 mm or greater were used for irrigation.

Subgingival Microbial Sample Collection

For each subject, subgingival plaque samples were collected for microbial analysis from each subject by examiners Y.K. and M.S. Sterile paper points were used to collect subgingival plaque samples from two sites with probing pocket depths of 5mm or more for each subject. The deepest non-adjacent sites in the quadrant were selected for microbial sampling. Microcentrifuge tubes were used to store the samples. Samples from each patient were pooled. The tubes were sealed and subsequently frozen at -80 C until further analyzed.

Protocol for Microbiological Sampling

Materials

Packet of paper points

Sterilize cotton pliers

Sterilize curette

Labelled tube for each subject

Cotton rolls

Transport box with ice

Collection

1. The two (2) sites were selected from one quadrant based on the periodontal chart

2. Sites were isolated with sterile cotton rolls

3. Supragingival plaque was removed from the selected tooth surface with a sterile curette

4. Test site was dried with air syringe

5. Sites were maintained dry using cotton rolls

5. One sterile paper point was inserted to the bottom of each pocket with cotton pliers

6. The paper point was left in place for 10 seconds. This was repeated with a second paper point at each site

7. All paper points were placed into labelled tubes

8. The tubes were placed on ice in a transport box

9. The box was transported to storage at -80 C

Irrigation:

All irrigation was completed by a single examiner (N.T.). All irrigation solutions were prepared fresh for each subject at the time of their SRP visit. Commercial bleach was diluted into water at a concentration of 0.05% using a micropipette. Three milliliter endodontic irrigation syringes were used to perform the irrigation. Irrigation was performed on all sites that exhibited 5mm of probing depth or greater within the selected quadrant.

Microbiome Analysis

The microbial samples were thawed and transferred to special transport vials (Explorer kit) and sent to uBiome (San Francisco, USA) for 16S rRNA sequencing and analysis. The 16S gene is

universal in bacteria, and it has both variable and conserved regions. The conserved regions are identical in bacteria. The 16S gene can be a marker for the identification of different species within a sample. Known gene sequences for strains are stored in databanks and unknown samples can be compared to them. [56] 16S rRNA gene sequence analysis is superior to more traditional methods of bacterial identification because it can recognize non-cultured bacteria and phenotypically unusual strains.[56]

The protocol for analysis and sampling was provided by uBiome. The 16S gene amplification is conducted by polymerase chain reaction (PCR) using an enzyme called Taq polymerase. The variable region within the 16S gene of the samples was amplified, and the copies were sequenced. The data was compared to online sequence databases for matches to known bacteria.[57]

Oral microbiome analyses included community relative abundance, phylogenetic and gene pathway differentials between treatment groups, principle component analyses, alpha and beta diversity, and random forest analyses to predict group status and to identify genetic features of the microbiome that relate to periodontal health outcomes.

Data Management

All study data were managed and stored in compliance with the IRB Data Protection Policy Research data were stored in the office of the Periodontics department and were kept confidential to the extent provided by law, with the principal and co-investigators having sole access to the

data. The information from this study may be published in scientific journals or presented at scientific meetings but the subjects' identity will be kept strictly confidential.

Study Completion

After study completion, all subjects continued with their periodontal care as needed at the UCSF Periodontology Clinic.

Statistical Methods

The recruitment of 25 subjects per arm would have been necessary to detect a difference between groups of 0.5 standard deviations, with 80% power at a significance level of p=0.05 (2-tailed). The proposed sample size would have allowed for the detection of a change of approximately 0.5 standard deviations in the microbiologic assessment and clinical data.

Quantitative data was collected from each arm of the study by study researchers in the clinic. Statistical analysis was primarily carried out at the individual level. Significance tests were performed to test for differences at baseline. Descriptive statistics were performed for continuous variables including the mean, standard deviation, range and the number of observations. The differences in means between the two groups were primarily analyzed by student's t-test.

III. Results

III. Results

A total of eight subjects completed the study and were included in the data analysis. Two subjects who met the inclusion and exclusion criteria ultimately declined to participate in the study due to reasons of increase in appointment time and lack of interest. Three additional subjects were enrolled and had completed the baseline examination, initial microbial sampling, and SRP with irrigation. They had not completed the study within the timeline provided. Thus, they were not included in the data analysis. Figure 3.1 illustrates the recruitment and enrollment of subjects. Of the eighteen patients screened, five were ineligible based on the inclusion criteria. Two patients declined to participate in the study. Three patients did not return for their final evaluation 4-6 weeks after the SRP appointment. Their final clinical measurements and microbial samples were not collected. The demographic data on the eight subjects who completed the study is listed in Table 3.1. Both the control group and the test group have four subjects. The smoking status of the subjects was equally distributed between the groups. The range of ages in the control group is larger, but the difference in mean age between the two groups is not statistically significant based on student's t-test (p value = 0.86), and there is no difference in variance based on F-test.

There was an overall decrease in the percentage of sites that had bleeding on probing in both groups at the final evaluation. The control group had a 27.53% decrease, and the test group had a 28.47% decrease in sites with BOP as shown in Table 3.2 and Table 3.3. The difference between the groups at the final evaluation was not statistically significant based on Student's t-test.

There was also an overall decrease in the percentage of sites that had plaque in both groups at the final evaluation. The control group had a 30.95% decrease, and the test group had a 33.33% decrease in sites with plaque as shown in Table 3.4 and Table 3.5. The difference between the groups at the final evaluation was not statistically significant based on Student's t-test.

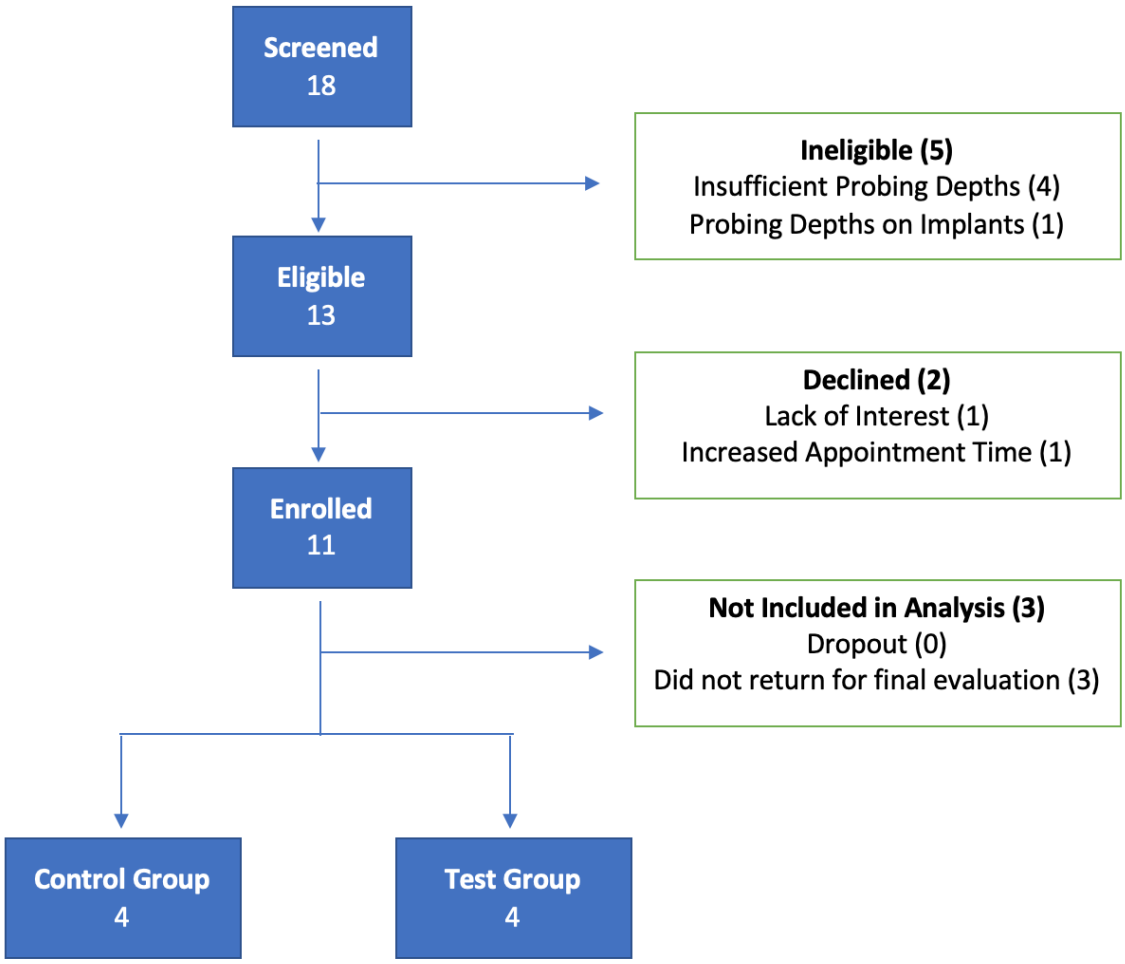

Figure 3.1 Flowchart describing Study Recruitment and Enrollment

Table 3.1 Study Demographics

Characteristics	Control (water)	Test (NaOCl)
n	4	4
mean age + SD (years)	58.25 ± 15.65	59.75 ± 6.13
• Range (years)	41 - 77	52 - 66
sex (female)	1	2
Smoking Status		
• smoker (≤10 cigarettes per day)	1	1
• former smoker	2	2
• non-smoker	1	1

Table 3.2 Percentage of Sites in Quadrant with Bleeding on Probing in Control Group

Subject	Baseline evaluation	Final evaluation	Change in bleeding (Final - Baseline)
A	100.00%	29.17%	-70.83%
B	2.38%	4.76%	2.38%
C	88.10%	21.43%	-66.67%
D	12.50%	37.50%	25.00%
Mean %	**49.24%**	**20.45%**	**-27.53%**

Table 3.3 Percentage of Sites in Quadrant with Bleeding on Probing in Test Group

Subject	Baseline evaluation	Final evaluation	Change in bleeding (Final - Baseline)
E	36.11%	13.89%	-22.22%
F	47.22%	19.44%	-27.78%
G	100.00%	83.33%	-16.67%
H	100.00%	52.78%	-47.22%
Mean %	**69.57%**	**40.58%**	**-28.47%**

Table 3.4 Percentage of Sites with Presence of Plaque in Quadrant in Control Group

Subject	Baseline evaluation	Final evaluation	Change in Plaque (Final - Baseline)
A	100.00%	16.67%	-83.33%
B	64.29%	64.29%	0.00%
C	50.00%	9.52%	-40.48%
D	0.00%	0.00%	0.00%
Mean (%)	**54.55%**	**26.52%**	**-30.95%**

Table 3.5 Percentage of Sites with Presence of Plaque in Quadrant in Test Group

Subject	Baseline evaluation	Final evaluation	Change in Plaque (Final - Baseline)
E	33.33%	19.44%	-13.89%
F	91.67%	30.56%	-61.11%
G	100.00%	100.00%	0.00%
H	100.00%	41.67%	-58.33%
Mean (%)	**80.43%**	**45.65%**	**-33.33%**

Table 3.6 and Table 3.7 show the mean pocket depth for each subject at baseline and final evaluation. There was an overall decrease in mean pocket depth in both groups. Three out of four subjects in each group had a decrease in mean pocket depth. There was a greater decrease in mean pocket depth in the control group.

Table 3.6 Mean Pocket Depth in Quadrant in Control Group

Subject	Baseline evaluation	Final evaluation	Change in Pocket Depth (Final - Baseline)
A	5.79	3.04	-2.75
B	2.83	2.31	-0.52
C	3.86	2.81	-1.05
D	3.21	3.50	0.29

Mean (mm)	**3.77**	**2.82**	**-1.01**

Table 3.8 and Table 3.9 show the mean clinical attachment loss for each subject at baseline and final evaluation. There was an overall decrease in mean clinical attachment loss in both groups. Three out of four subjects in each group had a decrease in mean pocket depth.

Table 3.7 Mean Pocket Depth in Quadrant in Test Group

Subject	Baseline evaluation	Final evaluation	Change in Pocket Depth (Final - Baseline)
E	3.78	2.67	-1.11
F	3.33	3.03	-0.31
G	5.00	4.53	-0.47
H	4.58	4.75	0.17
Mean (mm)	**4.14**	**3.71**	**-0.43**

Table 3.8 Mean Clinical Attachment Loss in Quadrant in Control Group

Subject	Baseline evaluation	Final evaluation	Change in Clinical Attachment Loss (Final - Baseline)
A	7.38	4.33	-3.04
B	2.50	2.05	-0.45
C	2.14	1.67	-0.48
D	3.58	3.88	0.29
Mean (mm)	**3.47**	**2.67**	**-0.80**

Table 3.9 Mean Clinical Attachment Loss in Quadrant in Test Group

Subject	Baseline evaluation	Final evaluation	Change in Clinical Attachment Loss (Final - Baseline)
E	4.97	3.81	-1.17
F	3.86	3.33	-0.53
G	3.33	2.87	-0.47

H	3.00	3.67	0.67
Mean (mm)	**3.81**	**3.44**	**-0.37**

Table 3.10 and Table 3.11 show the differences between the control group and the test group at baseline and final evaluation. The differences between the pocket depth, clinical attachment loss, bleeding on probing, and plaque are not statistically significant.

Table 3.10 Differences in Mean Baseline Clinical Characteristics of Study Groups by Per-Subject Analysis

Group	Control Group (mean ± SD)	Test Group (mean ± SD)	p value
Pocket Depth (mm)	3.77 ± 1.32	4.14 ± 0.76	p = 0.75
Clinical Attachment Loss (mm)	3.47 ± 2.40	3.81 ± 0.86	p = 0.93
Bleeding on Probing (%)	49.24 ± 50.41	69.57 ± 33.98	p = 0.53
Plaque (%)	54.55 ± 41.44	80.43 ± 32.19	p = 0.33

Table 3.11 Differences in Mean Final Clinical Characteristics of Study Groups by Per-Subject Analysis

Group	Control Group (mean ± SD)	Test Group (mean ± SD)	p value
Pocket Depth (mm)	2.82 ± 0.50	3.71 ± 1.05	p = 0.20
Clinical Attachment Loss (mm)	2.67 ± 1.32	3.44 ± 0.42	p = 0.56
Bleeding on Probing (%)	20.45 ± 13.94	40.58 ± 32.26	p = 0.33
Plaque (%)	26.52 ± 28.60	45.65 ± 35.89	p = 0.31

Figure 3.2 shows the distribution of pocket depth within each group at baseline and final evaluation. In the control group, 56.06% of all pockets are in the 1-3mm range. This increases to 83.33% at the final evaluation. 33.33% of the pockets in the control group are in the 4-6mm

range. This value decreases to 16.67% at the final evaluation. 10.61% of the pockets are greater than 6mm at the baseline evaluation and 0% at the final evaluation. In the test group, 50.0% of all pockets are in the 1-3mm range. This increases to 62.32% at the final evaluation. 37.68% of the pockets in the test group are in the 4-6mm range. This value decreases to 27.54% at the final evaluation. 12.34% of the pockets are greater than 6mm at the baseline evaluation and 10.15% at the final evaluation.

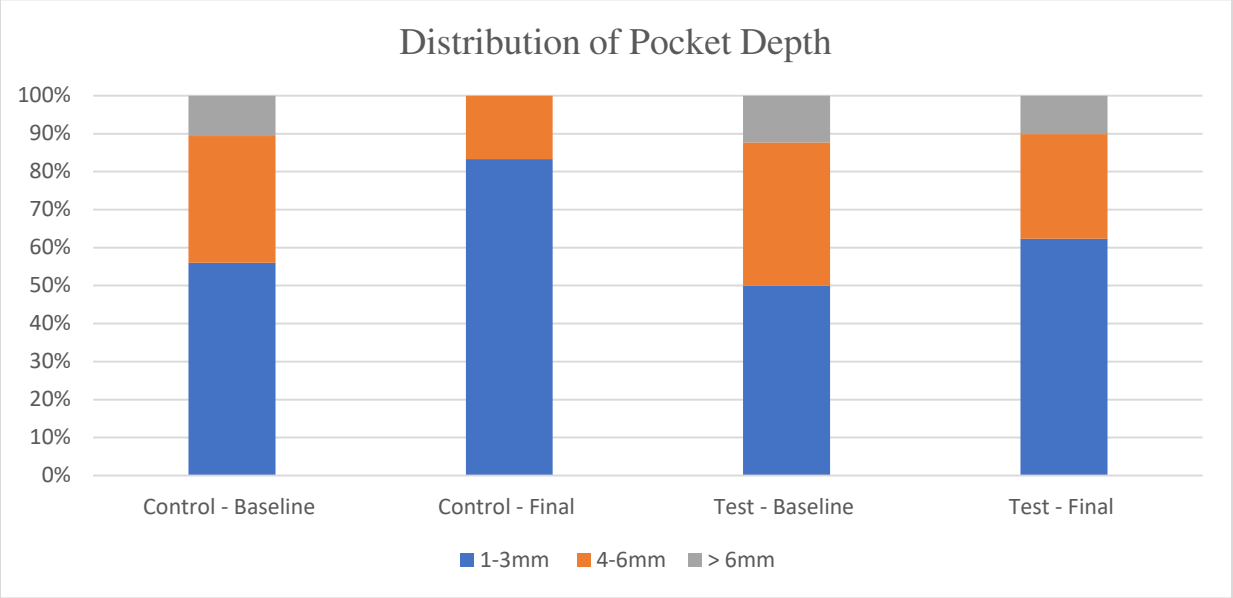

Figure 3.2 Distribution of Pocket Depth from Baseline to Final Evaluation for Control and Test Groups

The change in pocket depths was evaluated per-site in Table 3.12. There was a statistically significant decrease in pocket depth in both groups between baseline and final evaluation. Table 3.13 shows the mean number of sites per group that were 5mm or greater at baseline and final evaluation. All sites that were 5mm or greater at baseline evaluation were irrigated with either water or NaOCl. The mean number of sites decreases for both groups at the final evaluation.

Table 3.12 Per Site Analysis of Pocket Depths Baseline and Final Evaluation

Group		Baseline	Final	p value
Control	**n**	132	132	
	Mean + SD (mm)	3.78 ± 1.62	2.86 ± 0.93	*p= 3.561E-13
Test	**n**	138	138	
	Mean + SD (mm)	4.13 ± 1.99	3.73 ± 2.24	*p = 0.00069

*Statistical Significance $p < 0.05$

Table 3.13 Sites with Pocket Depths 5mm or Greater at Baseline and Final Evaluation

Group	Data	Baseline	Final
Control	mean + SD (sites)	8.75 ± 7.27	2 ± 1.41
	Range (sites)	2 - 16	1 - 4
Test	mean + SD (sites)	12.5 ± 6.24	7.25 ± 6.24
	Range (sites)	6 - 21	1 - 14

Microbiome Analysis

The community relative abundance of the phyla among microbial samples were analyzed in Figure 3.3. The relative abundance of the *Bacteroidetes* phylum decreased in both groups from baseline to final evaluation. The relative abundance of *Fusobacterium* increased in both groups from baseline to final evaluation. The relative abundance of *Actinobacteria* increased in the control group, but it decreased in the experimental group from baseline to final evaluation. Due to the small sample size, the differences did not achieve statistical significance.

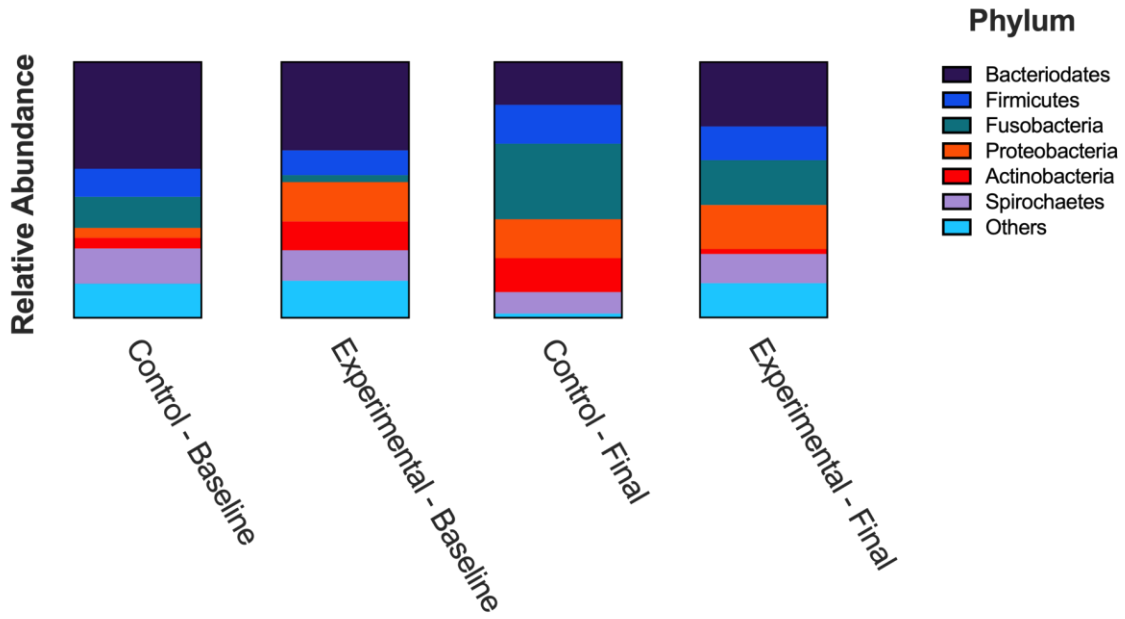

Figure 3.3 Relative Phyla Abundance from Baseline to Final Evaluation for Control and Experimental Groups

The diversity of the microbial samples was measured with Simpson's diversity index in Figure 3.4 and the Shannon diversity index in Figure 3.5. Simpson's diversity index incorporates the number and abundance of each species within the samples.[58] Similarly, the Shannon diversity index incorporates the abundance and the evenness of the species.[59] Both indices showed that the experimental group was less diverse at baseline, but the diversity increased at the final evaluation. The diversity indices of control group remained relatively unchanged between baseline and final evaluations.

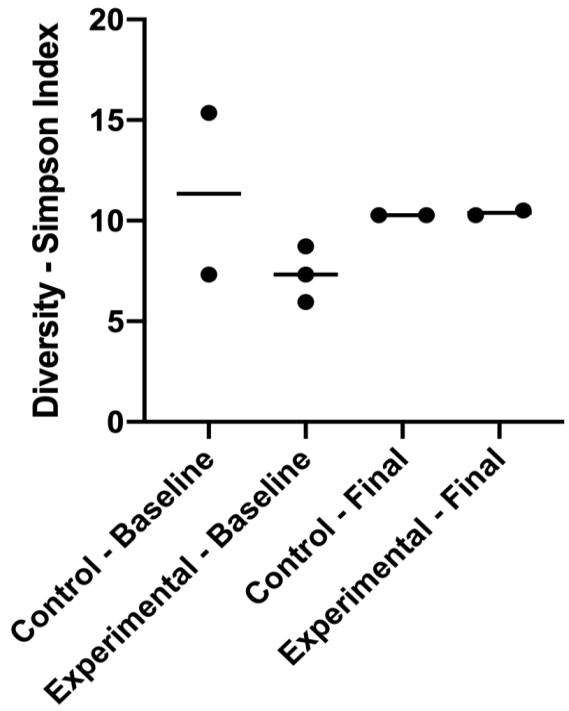

Figure 3.4 Simpson's Diversity Index for Species in Control and Experimental Groups at Baseline and Final Evaluation

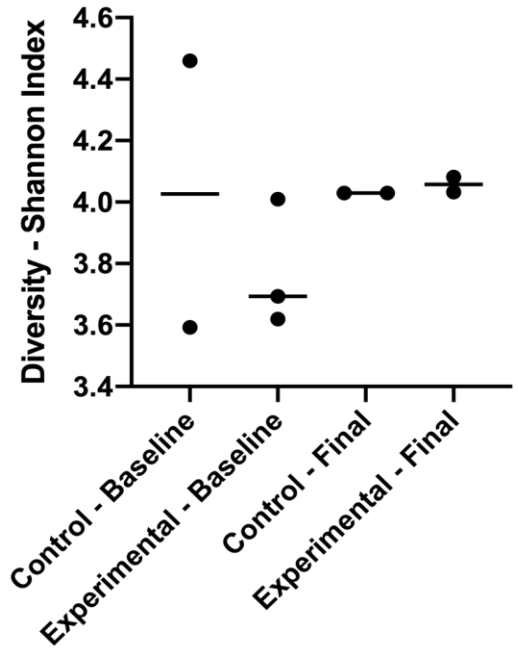

Figure 3.5. Shannon Diversity Index in Control and Experimental Groups at Baseline and Final Evaluation

The richness of the samples is analyzed in Figure 3.6. The richness of species is an important factor in evaluating the health of the system. Higher richness is generally considered beneficial for the ecosystem. The richness increases for the experimental group at final evaluation, and slightly decreases for the control group.

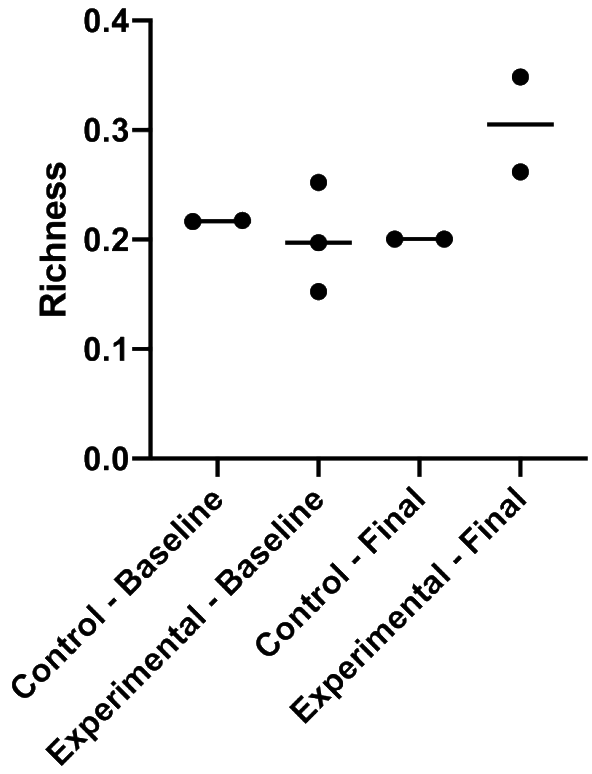

Figure 3.6. Species Richness in Control and Experimental Groups at Baseline and Final Evaluation

Analysis was performed on the genera of some known periodontal pathogens that were identified in the samples. The percent composition of *Porphyromonas* was analyzed in Figure 3.7. The percent of *Porphyromonas* within the control samples decreased between baseline and final evaluation. The percent of *Porphyromonas* within the experimental samples was slightly higher at baseline. The percent also decreased between baseline and final evaluation in the experimental

group. The experimental group had a greater percent of *Porphyromonas* at the final evaluation compared to the control group. The percent composition of *Aggregatibacter* was analyzed in Figure 3.8. The percent of *Aggregatibacter* within the control group increased by less than 1% between the baseline and final evaluation. The percent of *Aggregatibacter* within the experimental group increased by 1.5% between the baseline and final evaluation. The percent of *Aggregatibacter* in the experimental group remained higher relative to the control group at both time points. The percent composition of *Tannerella* was analyzed in Figure 3.9. The control group decreases from 3.5% to 0.5% at final evaluation, while the experimental group was relatively unchanged.

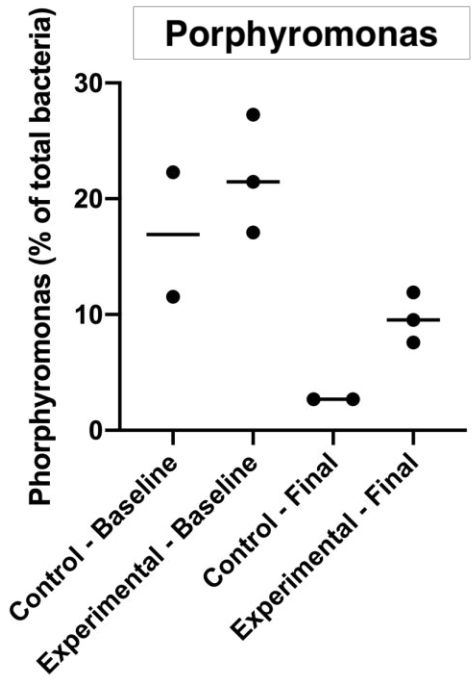

Figure 3.7. *Porphyromonas* Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation

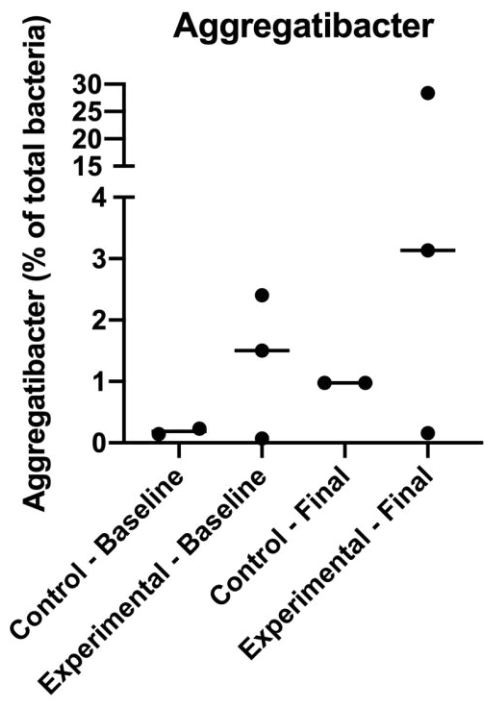

Figure 3.8. ***Aggregatibacter*** **Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation**

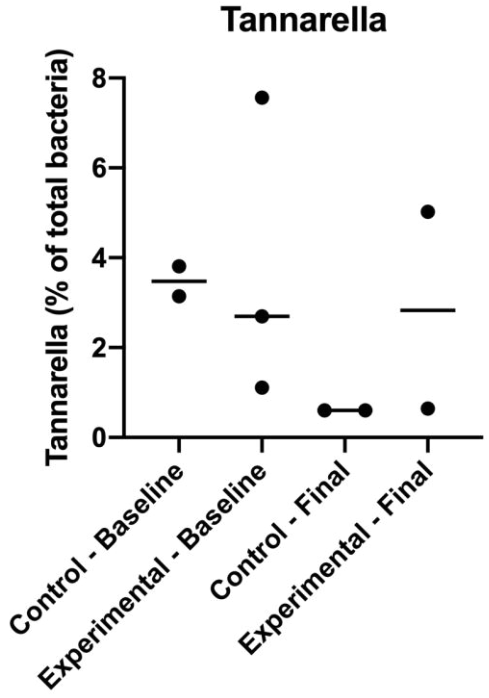

Figure 3.9. ***Tannerella*** **Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation**

The percent composition of *Haemophilus*, *Streptococci, and Veillonella* were analyzed in Figures 3.10, 3.11, and 3.12, respectively. These three genera are considered commensals. There was a greater increase in the percent composition of this genera in the experimental group. The percent composition for all three genera was greater in the experimental group at final evaluation.

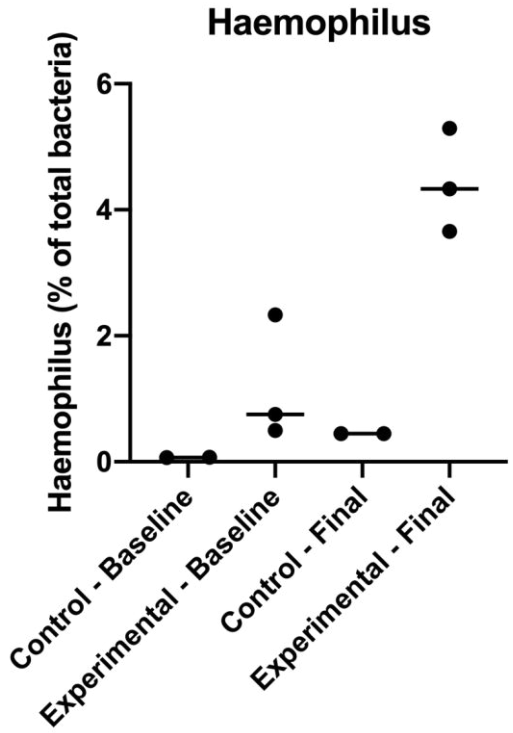

Figure 3.10. *Haemophilus* Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation

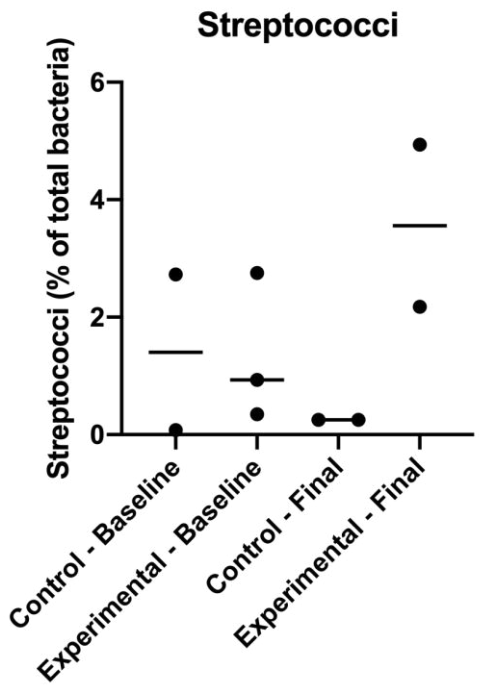

Figure 3.11. ***Streptococci*** **Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation**

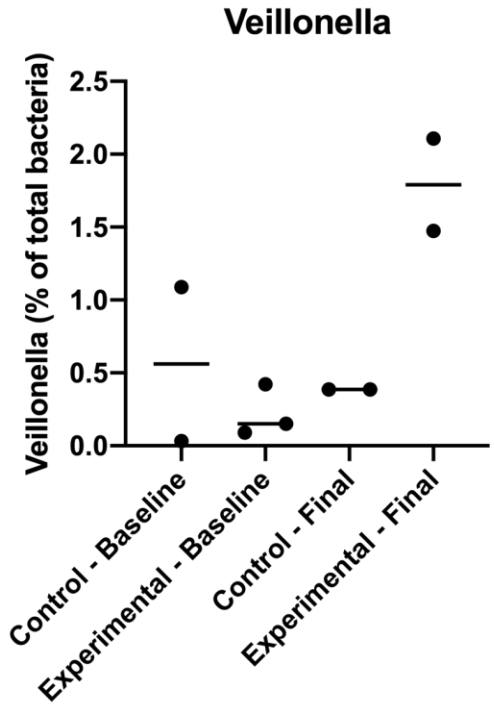

Figure 3.12. ***Veillonella*** **Percent Composition within Total Bacteria for Control and Experimental Groups at Baseline and Final Evaluation**

Adverse reactions

One subject from the NaOCl irrigation group reported temporary post-procedural pain and sensitivity on the quadrant one week following SRP with irrigation. It was unclear whether the source of the pain was the SRP procedure or the NaOCl irrigation. The subject did not complain of persisting symptoms at the final evaluation.

IV. Discussion

IV. Discussion

Based on the results of the present study, there is no statistically significant difference in pocket depth and clinical attachment loss at 4-6 weeks following scaling and root planing between the group that received NaOCl irrigation and that which received water irrigation. Both groups had an overall decrease in mean pocket depth and clinical attachment loss at the final evaluation, which highlights the effect of scaling and root planing on periodontitis. The effect of the NaOCl irrigation is not seen in the present study pocket depth and clinical attachment loss. A statistically significant difference was only seen when the analysis was conducted for each site. Both groups showed a decrease in pocket depth and clinical attachment loss at the final evaluation. Analysis of the bleeding on probing and plaque showed a decrease in both groups at the final evaluation. Although both groups improved in all the periodontal parameters by the final evaluation, the mean values in test group were consistently higher at both baseline and final evaluation. Although the differences at baseline were not statistically significant in this small sample size, there is a possibility that the test group was worse overall at baseline. 26.5% of the sites in the control group had a probing depth of 5mm or greater compared to 36.2% of the sites in the test group. The success of periodontal therapy is influenced by patient compliance with oral hygiene instruction and ability to perform oral hygiene procedures.[60] The test group had poorer plaque control and more sites with bleeding on probing at the baseline and final evaluation compared to the control group. This study would have benefited from a larger sample size to account for these differences.

One issue with the use of irrigation is that the effect is transient. A sustained release system can maintain a certain concentration of the antimicrobial in the gingival crevice over a period of

time. A systematic review by Bonito et al. found an improvement in pocket depth and clinical attachment gain with the use of adjunctive locally-delivered antimicrobials during scaling and root planing. The clinical difference for probing depth ranged from 0.1-0.5mm in favor of the added antimicrobials, but the effect was generally transient. The antimicrobials were incorporated into chips, fibers, or microspheres, which allowed for sustained release over a finite period of time.[61] NaOCl irrigation did not have a significant effect in the present study, but a possibility for further exploration can involve a stabilized form in a sustained release product. Other studies on NaOCl use in periodontitis patients showed a greater effect with the regular use of oral rinses.[39,62] Incorporating a rinse into patient homecare may be more effective because of the increased frequency of use. However, patient compliance and the ability of patients to correctly dilute the rinse each day must be taken into consideration. The concentration used in this study was among the lowest reported in other studies at 0.05%. It is possible that the concentration was too low to have an effect on the periodontal measurements in this study.

The analysis of the microbiome revealed some trends, but statistically significant differences in the data could not be determined with the small sample size. The *Bacteriodetes* phylum includes *P. Gingivalis*, which is a known periodontal pathogen. The sample data shows a decrease in relative abundance of *P. Gingivalis* at the final evaluation. This indicates that both groups may have benefitted from the SRP, and the use of NaOCl did not increase the abundance of this known pathogen. There were inherent differences in the relative abundance of phyla between the groups at baseline, therefore, it is difficult to ascribe the changes at final evaluation to either the SRP or the irrigation. A larger sample size can provide better insight into the changes in abundance.

Based on the Shannon diversity index and the Simpson diversity index, the experimental group showed less diversity at baseline in comparison to the control group. At the final evaluation, the diversity increased in the experimental group to levels similar to the control group. This may be attributed to the use of NaOCl, which is a known for its bactericidal effect. If part of the biofilm was eliminated at the time of irrigation, there may have been a shift in the diversity as the biofilm reformed. However, this cannot be confirmed by the limited data available. The diversity of species is an important component of ecologic systems. Higher diversity is usually desired for any ecosystem.

Study Limitations

One of the major issues with the present study was the small sample size. Four subjects were enrolled in each group, which resulted in a lack of power. Only short-term effects were evaluated, so the long-term effects, if any, are unknown. There was also no calibration conducted among residents who performed the baseline and final evaluation.

Summary

The present study showed no difference between irrigation with 0.05% NaOCl and water. The effect scaling and root planing on the clinical parameters of periodontal disease is seen.

Future Direction

The continuation of this study to incorporate a larger sample size may further elucidate the effect of NaOCl irrigation. The microbiome analysis showed some trends, which can be further explored with a larger sample size. NaOCl irrigation did not have a significant effect in the

present study, but a possibility for further exploration can involve a stabilized form in a sustained release product. Developing a device for patient use that facilitates the dilution of NaOCl to a safe concentration for rinsing can be explored.

Funding

This study was funded by the UCSF Division of Periodontology.

References

References

1. Eke, P. I., Dye, B. A., Wei, L., Thornton-Evans, G. O. & Genco, R. J. Prevalence of periodontitis in adults in the united states: 2009 and 2010. *J. Dent. Res.* (2012). doi:10.1177/0022034512457373
2. Tonetti, M. S., Jepsen, S., Jin, L. & Otomo-Corgel, J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *J. Clin. Periodontol.* (2017). doi:10.1111/jcpe.12732
3. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* (2016). doi:10.1016/S0140-6736(16)31678-6
4. Murray, C. J. L. *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet* (2012). doi:10.1016/S0140-6736(12)61689-4
5. Kassebaum, N. J. *et al.* Global burden of severe periodontitis in 1990-2010: A systematic review and meta-regression. *Journal of Dental Research* (2014). doi:10.1177/0022034514552491
6. Chapple, I. L. C. *et al.* Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J. Clin. Periodontol.* (2017). doi:10.1111/jcpe.12685
7. LÖE, H. Periodontal diseases: a brief historical perspective. *Periodontol. 2000* (1993). doi:10.1111/j.1600-0757.1993.tb00215.x
8. J, M. & I, C. Molecular Aspects of the Pathogenesis of Periodontitis. *Periodontol. 2000*

(2015). doi:10.1111/PRD.12104

9. Page, R. C. & Schroeder, H. E. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab. Invest.* (1976).

10. Socransky, S. S. Criteria for the infectious agents in dental caries and periodontal disease. *J. Clin. Periodontol.* (1979). doi:10.1111/j.1600-051X.1979.tb02114.x

11. Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C. & Kent, R. L. Microbial complexes in subgingival plaque. *J. Clin. Periodontol.* (1998).

12. Hajishengallis, G., Darveau, R. P. & Curtis, M. A. The keystone-pathogen hypothesis. *Nature Reviews Microbiology* (2012). doi:10.1038/nrmicro2873

13. Theilade, E. The non-specific theory in microbial etiology of inflammatory periodontal diseases. *J. Clin. Periodontol.* (1986). doi:10.1111/j.1600-051X.1986.tb01425.x

14. Listgarten, M. A. The role of dental plaque in gingivitis and periodontitis. *J. Clin. Periodontol.* (1988). doi:10.1111/j.1600-051X.1988.tb01019.x

15. Smiley, C. J. *et al.* Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *Journal of the American Dental Association* (2015). doi:10.1016/j.adaj.2015.01.028

16. Needleman, I., Nibali, L. & Di Iorio, A. Professional mechanical plaque removal for prevention of periodontal diseases in adults - Systematic review update. *Journal of Clinical Periodontology* (2015). doi:10.1111/jcpe.12341

17. Tonetti, M. S. *et al.* Principles in prevention of periodontal diseases: Consensus report of group 1 of the 11th European Workshop on Periodontology on effective prevention of periodontal and peri-implant diseases. in *Journal of Clinical Periodontology* (2015). doi:10.1111/jcpe.12368

18. Deas, D. E., Moritz, A. J., Sagun, R. S., Gruwell, S. F. & Powell, C. A. Scaling and root planing vs. conservative surgery in the treatment of chronic periodontitis. *Periodontology 2000* (2016). doi:10.1111/prd.12114

19. Smiley, C. J. *et al.* Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *J. Am. Dent. Assoc.* (2015). doi:10.1016/j.adaj.2015.01.026

20. Haffajee, A. D. The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *J. Clin. Periodontol.* (1997). doi:10.1111/j.1600-051X.1997.tb00765.x

21. van Winkelhof, A. J., van der Velden, U. & de Graaff, J. Microbial succession in recolonizing deep periodontal pockets after a single course of supra- and subgingival debridement. *J. Clin. Periodontol.* (1988). doi:10.1111/j.1600-051X.1988.tb01004.x

22. Greenstein, G. Periodontal Response to Mechanical Non-Surgical Therapy: A Review. *J. Periodontol.* (2012). doi:10.1902/jop.1992.63.2.118

23. Waerhaug, J. Healing of the Dento-Epithelial Junction Following Subgingival Plaque Control: II: As Observed on Extracted Teeth. *J. Periodontol.* **49**, 119–134 (1978).

24. Rabbani, G. M., Ash, M. M. & Caffesse, R. G. The Effectiveness of Subgingival Scaling and Root Planing in Calculus Removal. *J. Periodontol.* (2010). doi:10.1902/jop.1981.52.3.119

25. Jones, S. J., Lozdan, J. & Boyde, A. Tooth surfaces treated in situ with periodontal instruments. Scanning electron microscopic studies. *Br. Dent. J.* (1972). doi:10.1038/sj.bdj.4802798

26. Bower, R. C. Furcation Morphology Relative to Periodontal Treatment: Furcation

Entrance Architecture. *J. Periodontol.* (1979). doi:10.1902/jop.1979.50.1.23

27. Caffesse, R. G., Sweeney, P. L. & Smith, B. A. Scaling and root planing with and without periodontal flap surgery. *J. Clin. Periodontol.* (1986). doi:10.1111/j.1600-051X.1986.tb01461.x

28. Lindhe, J., Socransky, S. S., Nyman, S., Haffajee, A. & Westfelt, E. "Critical probing depths" in periodontal therapy. *J. Clin. Periodontol.* (1982). doi:10.1111/j.1600-051X.1982.tb02099.x

29. Chapple, I. L. C. *et al.* Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. in *Journal of Clinical Periodontology* (2018). doi:10.1111/jcpe.12940

30. Mohammadi, Z. Sodium hypochlorite in endodontics: an update review. *Int. Dent. J.* (2008).

31. Gray, M. J., Wholey, W.-Y. & Jakob, U. Bacterial Responses to Reactive Chlorine Species. *Annu. Rev. Microbiol.* (2013). doi:10.1146/annurev-micro-102912-142520

32. Arias-Moliz, M. T., Ordinola-Zapata, R., Baca, P., Ruiz-Linares, M. & Ferrer-Luque, C. M. Antimicrobial activity of a sodium hypochlorite/etidronic acid irrigant solution. *J. Endod.* (2014). doi:10.1016/j.joen.2014.07.031

33. Vianna, M. E. *et al.* In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* (2004). doi:10.1016/S1079-2104(03)00360-3

34. Clegg, M. S., Vertucci, F. J., Walker, C., Belanger, M. & Britto, L. R. The Effect of Exposure to Irrigant Solutions on Apical Dentin Biofilms In Vitro. *J. Endod.* (2006).

doi:10.1016/j.joen.2005.07.002

35. Baumgartner, J. C. & Ibay, A. C. The chemical reactions of irrigants used for root canal debridement. *J. Endod.* (1987). doi:10.1016/S0099-2399(87)80153-X

36. Stojicic, S., Zivkovic, S., Qian, W., Zhang, H. & Haapasalo, M. Tissue dissolution by sodium hypochlorite: Effect of concentration, temperature, agitation, and surfactant. *J. Endod.* (2010). doi:10.1016/j.joen.2010.06.021

37. De Nardo, R., Chiappe, V., Gómez, M., Romanelli, H. & Slots, J. Effects of 0.05% sodium hypochlorite oral rinse on supragingival biofilm and gingival inflammation. *Int. Dent. J.* (2012). doi:10.1111/j.1875-595X.2011.00111.x

38. Medical Technology Assessment Directory: A Pilot Reference to Organizations, Assessments, and Information Resources. *Ann. Intern. Med.* (2013). doi:10.7326/0003-4819-109-1-87_3

39. Galván, M. *et al.* Periodontal effects of 0.25% sodium hypochlorite twice-weekly oral rinse. A pilot study. *J. Periodontal Res.* (2014). doi:10.1111/jre.12151

40. Jurczyk, K., Nietzsche, S., Ender, C., Sculean, A. & Eick, S. In-vitro activity of sodium-hypochlorite gel on bacteria associated with periodontitis. *Clin. Oral Investig.* (2016). doi:10.1007/s00784-016-1711-9

41. Bizzarro, S., Van der Velden, U. & Loos, B. G. Local disinfection with sodium hypochlorite as adjunct to basic periodontal therapy: a randomized controlled trial. *J. Clin. Periodontol.* (2016). doi:10.1111/jcpe.12578

42. Theilade, E., Wright, W. H., Jensen, S. B. & Löe, H. Experimental gingivitis in man: II. A Longitudinal Clinical and Bacteriological Investigation. *J. Periodontal Res.* (1966). doi:10.1111/j.1600-0765.1966.tb01842.x

43. Rams, T. E. & Slots, J. Local delivery of antimicrobial agents in the periodontal pocket. *Periodontol. 2000* (1996). doi:10.1111/j.1600-0757.1996.tb00072.x

44. Pitcher, G. R., Newman, H. N. & Strahan, J. D. Access to subgingival plaque by disclosing agents using mouthrinsing and direct irrigation. *J. Clin. Periodontol.* (1980). doi:10.1111/j.1600-051X.1980.tb01972.x

45. Braun, R. E. & Ciancio, S. G. Subgingival Delivery by an Oral Irrigation Device. *J. Periodontol.* (2012). doi:10.1902/jop.1992.63.5.469

46. Eakle, W. S., Ford, C. & Boyd, R. L. Depth of penetration in periodontal pockets with oral irrigation. *J. Clin. Periodontol.* (1986). doi:10.1111/j.1600-051X.1986.tb01412.x

47. Soh, L. L., Newman, H. N. & Strahan, J. D. Effects of subgingival chlorhexidine irrigation on periodontal inflammation. *J. Clin. Periodontol.* (1982). doi:10.1111/j.1600-051X.1982.tb01223.x

48. Hauman, C. H. J. & Love, R. M. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. *Int. Endod. J.* (2003).

49. Kaufman, A. Y. & Keila, S. Hypersensitivity to sodium hypochlorite. *J. Endod.* (1989). doi:10.1016/S0099-2399(89)80241-9

50. Çaliskan, M. K., Turkun, M. & Alper, S. Allergy to sodium hypochlorite during root canal therapy: acase report. *Int. Endod. J.* (1994). doi:10.1111/j.1365-2591.1994.tb00247.x

51. Armitage, G. C. Development of a classification system for Periodontal Disease. *Ann Periodontol* (1999). doi:10.1097/00006199-197401000-00014

52. American Society of Anesthesiologists. American Society of Anesthesiologists: ASA Physical Status Classification System. *American Society of Anesthesiologists Web site.*

Internet (2014).

53. American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions. *J. Periodontol.* (2015). doi:10.1902/jop.2015.157001

54. Lang, N. P., Joss, A., Orsanic, T., Gusberti, F. A. & Siegrist, B. E. Bleeding on probing. A predictor for the progression of periodontal disease? *J. Clin. Periodontol.* (1986). doi:10.1111/j.1600-051X.1986.tb00852.x

55. Ainamo, J. & Bay, I. Problems and proposals for recording gingivitis and plaque. *Int. Dent. J.* (1975).

56. Clarridge, J. E. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews* (2004). doi:10.1128/CMR.17.4.840-862.2004

57. The Best of Both Worlds: Precision Sequencing™ - uBiome. Available at: https://ubiome.com/blog/post/best-worlds-precision-sequencing/. (Accessed: 13th June 2019)

58. Simpson, E. H. Measurement of diversity [16]. *Nature* (1949). doi:10.1038/163688a0

59. Spellerberg, I. A. N. F. & Fedor, P. J. Spellerberg_2003_A Tribute to Claude Shannon (1916-2001) and a Plea for More Rigorous Use of Species Richness. *Glob. Ecol. Biogeogr.* (2003).

60. Baker, K. A. The role of dental professionals and the patient in plaque control. *Periodontol. 2000* (1995). doi:10.1111/j.1600-0757.1995.tb00048.x

61. Bonito, A. J., Lux, L. & Lohr, K. N. Impact of Local Adjuncts to Scaling and Root Planing in Periodontal Disease Therapy: A Systematic Review. *J. Periodontol.* (2005).

doi:10.1902/jop.2005.76.8.1227

62. Gonzalez, S. *et al.* Gingival bleeding on probing: Relationship to change in periodontal pocket depth and effect of sodium hypochlorite oral rinse. *J. Periodontal Res.* (2015). doi:10.1111/jre.12219

Publishing Agreement

It is the policy of the University to encourage the distribution of all theses, dissertations, and manuscripts. Copies of all UCSF theses, dissertations, and manuscripts will be routed to the library via the Graduate Division. The library will make all theses, dissertations, and manuscripts accessible to the public and will preserve these to the best of their abilities, in perpetuity.

Please sign the following statement:

I hereby grant permission to the Graduate Division of the University of California, San Francisco to release copies of my thesis, dissertation, or manuscript to the Campus Library to provide access and preservation, in whole or in part, in perpetuity.

Author Signature

09/03/19

Date