University of Nebraska Medical Center
BREAKTHROUGHS FOR LIFE.

University of Nebraska Medical Center
DigitalCommons@UNMC

Theses & Dissertations
Graduate Studies

Summer 8-14-2015

# Effect of Chemical Pocket Disinfection as an Adjunct to Non-Surgical Maintenance Therapy of Inflamed Periodontal Pockets

Chad Riggs
*University of Nebraska Medical Center*

Tell us how you used this information in this short survey.

Follow this and additional works at: https://digitalcommons.unmc.edu/etd

Part of the Periodontics and Periodontology Commons

## Recommended Citation

Riggs, Chad, "Effect of Chemical Pocket Disinfection as an Adjunct to Non-Surgical Maintenance Therapy of Inflamed Periodontal Pockets" (2015). *Theses & Dissertations*. 5.
https://digitalcommons.unmc.edu/etd/5

This Thesis is brought to you for free and open access by the Graduate Studies at DigitalCommons@UNMC. It has been accepted for inclusion in Theses & Dissertations by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

**EFFECT OF CHEMICAL POCKET DISINFECTION AS AN ADJUNCT TO NON-SURGICAL MAINTENANCE THERAPY OF INFLAMED PERIODONTAL POCKETS**

by

**Chad M. Riggs, D.D.S.**

A THESIS

Presented to the Faculty of

the University of Nebraska Graduate College

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

Medical Sciences Interdepartmental Area
Graduate Program

(Oral Biology)

Under the Supervision of Dr. Wayne B. Kaldahl

University of Nebraska Medical Center
Omaha, Nebraska

June, 2015

Advisory Committee:

Dr. Richard A. Reinhardt, D.D.S., M.S., Ph.D.

Dr. David H. Shaw, M.S., Ph.D.

Dr. Matthew R. Byarlay, D.D.S., M.S.

Dr. Nagamani Narayana, D.M.D., M.S

**ACKNOWLEDGEMENTS**

I was unsure what to expect when applying to the master's program during my first year of residency. I knew I wanted to engage in research that would significantly contribute to the field of periodontics. I had hoped it would give insight into the volumes of literature I would encounter during my residency program and help to stay abreast of the advances in my specialty. Now as I conclude my studies in Lincoln, Nebraska three years later, I am happy to report my experience far exceeded my expectations. I am profoundly grateful for the generous and expert help I have received in completing my research project and for the enriching relationships I have developed with the stellar faculty and staff of this university who advised me on my project.

First and foremost, I would like to thank Dr. Wayne Kaldahl for being my research mentor. I am grateful for the countless times I knocked on his office door, interrupting his train of thought, only to have him welcome me in as he sat amongst his towering mounds of papers and projects. His guidance was crucial to the completion of my project and our association has deeply enriched my professional and personal life. He is one of the most humble, patient, and competent men with whom I have had the pleasure to work.

I am indebted to all the members of my research committee for their time and commitment in helping me succeed. Dr. Richard Reinhardt provided his incredible knowledge in the analysis of the crevicular fluid samples and demystified the hazy world of cytokines for me. Dr. Matthew Byarlay administered treatment to all of the study patients. Dr. David Shaw and Dr. Nagamani Narayana gave thoughtful insight and guidance in putting together the research protocol. Dr. David Marx was crucial in analyzing the data with his statistical expertise.

Additionally, I acknowledge Mrs. Marian Schmid, who was essential in processing lab samples on several early mornings; Mrs. Megan Christiansen, who took time during her

appointments to effectively and successfully recruit all of the patients in this study; and Ms. Betty Shestak who recorded each and every measurement and provided all materials necessary for each appointment.

I also recognize all of the patients who were willing to participate in the study, enhancing my educational experience as well as advancing the field of periodontics. Without them, this study could not have happened. And I thank the UNMC College of Dentistry Periodontal Development program, with the approval of Dr. Bruce Bavitz, for funding this research.

Finally, I gratefully acknowledge the support I received daily from my loving wife, Lyndsay, and my children who make life so rewarding and give me a deeper perspective on life.

**EFFECT OF CHEMICAL POCKET DISINFECTION AS AN ADJUNCT TO NON-SURGICAL MAINTENANCE THERAPY OF INFLAMED PERIODONTAL POCKETS**

Chad M. Riggs, D.D.S., M.S.

University of Nebraska, 2015

**Advisor:** Wayne B. Kaldahl, D.D.S.

**Purpose:** Scaling and root planing with adjunctive chemical pocket disinfection (SRP+C) utilizing sodium hypochlorite solution has been used to treat inflamed pockets of periodontal maintenance patients for many years, without evidence of its benefits. The primary objective was to determine if SRP+C is more effective than scaling and root planing alone (SRP) in improving clinical outcomes. The secondary objective was to compare the effect of SRP+C and SRP on pro-inflammatory IL-1β, anti-inflammatory IL-1ra, and anti-inflammatory index (IL-1ra/IL-1β ratio) found in gingival crevicular fluid (GCF).

**Materials and Methods:** Pockets (≥ 5 mm and BOP) of 31 maintenance patients were included. Test (SRP+C; 41 sites) and control (SRP; 43 sites) therapies were randomly administered. Clinical measurements and GCF samples were collected at baseline and 3-months post-therapy. Cytokine levels were determined and all data analyzed.

**Results:** Both SRP+C and SRP resulted in significant improvements of all clinical outcomes with no differences between therapies. There were no differences between therapies in IL-1β and anti-inflammatory index. IL-1ra was greater in SRP+C than SRP post-therapy ($P = 0.007$). When the results of both therapies were combined, the anti-inflammatory index was greater ($P = 0.02$) with a trend in greater PD reduction ($P = 0.0552$) in sites where bleeding on probing (BOP) resolved compared to unresolved sites.

**Conclusions:** The addition of sodium hypochlorite to scaling and root planing did not improve clinical parameters in inflamed pockets of periodontal maintenance patients, but increased anti-inflammatory IL-1ra.

**TABLE OF CONTENTS**

ACKNOWLEDGEMENTS ..... i

ABSTRACT ..... vii

TABLE OF CONTENTS ..... iv

LIST OF FIGURES/TABLES ..... vi

LIST OF ABBREVIATIONS ..... vii

INTRODUCTION ..... 1

LITERATURE REVIEW: CHEMICAL POCKET DISINFECTION/CHEMICAL CURETTAGE ..... 4

LITERATURE REVIEW: PERIODONTITIS, INTERLEUKEN-1β, AND INTERLEUKIN-1ra ..... 11

MATERIALS AND METHODS ..... 18

*Overview of study design* ..... 18
*Patient selection* ..... 20
*Data collection* ..... 21
*Treatment protocol* ..... 23
*Analysis of GCF samples* ..... 25
*Statistical analyses* ..... 28

RESULTS ..... 29

*Examiner calibration* ..... 29
*Patient characteristics* ..... 29
*Clinical outcomes* ..... 30
*Cytokine outcomes* ..... 31
*Outcomes based on BOP resolution* ..... 33

DISCUSSION ..... 35

CONCLUSIONS ..... 41

BIBLIOGRAPHY ..... 42

Appendix A: Plaque index ..... 53

Appendix B: Concentrated sodium hypochlorite formula ..... 54

Appendix C: Raw clinical data for SRP+C ........ 55

Appendix D: Raw clinical data for SRP ........ 56

Appendix E: Raw IL-1β and IL-1ra cytokine data in diseased sites treated with SRP+C ........ 57

Appendix F: Raw IL-1β and IL-1ra cytokine data in diseased sites treated with SRP ........ 58

Appendix G: Raw IL-1β and IL-1ra cytokine data in healthy sites ........ 59

Appendix H: Patient research participation forms and consent ........ 59

**LIST OF FIGURES/TABLES**

Figure 1: Flow of study design ........ 19

Figure 2: Modified Orban medicament loop used to deliver chemical solutions ........ 24

Table 1: SRP+C patient characteristics at baseline ........ 29

Table 2: SRP patient characteristics at baseline ........ 30

Table 3: Clinical outcomes ........ 30

Table 4: IL-1β levels........ 31

Table 5: IL-1ra levels ........ 31

Table 6: Anti-inflammatory index (IL-1ra/IL-1β ratio) ........ 32

Table 7: Clinical outcomes by BOP resolution ........ 33

Table 8: IL-1β levels by BOP resolution........ 33

Table 9: IL-1ra levels by BOP resolution ........ 33

Table 10: Anti-inflammatory index (IL-1ra/IL-1β ratio) by BOP resolution ........ 34

**LIST OF ABBREVIATIONS**

| | |
|---|---|
| BOP | bleeding on probing |
| CAL | clinical attachment level |
| ELISA | enzyme-linked immunosorbent assay |
| GCF | gingival crevicular fluid |
| H | healthy |
| IL-1β | interleukin-1 beta |
| IL-1ra | interleukin-1 receptor antagonist |
| NSAID | non-steroidal anti-inflammatory drug |
| PD | probing depth |
| PI | plaque index |
| REC | gingival recession |
| SRP | scaling and root planing alone (control therapy) |
| SRP+C | scaling and root planing with adjunctive chemical pocket disinfection (test therapy) |

**INTRODUCTION**

Chronic periodontitis is an inflammatory disease affecting the attachment apparatus supporting the teeth (AAP Parameters of Care 2000). This apparatus is termed the periodontium and includes the gingiva, periodontal ligament, cementum, and alveolar bone. According to the Center for Disease Control and Prevention in 2012, approximately half of adults aged 30 or over have some form of periodontal disease (Eke et al. 2015) and its prevalence and severity increases with age (Lindhe et al. 1999).

Periodontal disease may clinically manifest as gingival erythema, pain, increased gingival crevicular fluid (GCF), tooth mobility, bleeding on probing (BOP), clinical attachment level (CAL) loss, increased probing depth (PD), suppuration, and gingival recession (REC). If left untreated, the tooth-supporting bone and gingival tissue may progressively break down and result in tooth loss (AAP Position Paper 1999).

Diagnosis of chronic periodontitis is made based on the observation of traditional clinical parameters such as: presence or absence of signs of inflammation, severity of attachment loss and bone destruction, pocketing, extent and pattern of involved teeth, medical and dental histories, pain, ulceration, and amount of plaque and calculus present (AAP Position Paper 2003). Chronic periodontitis must be differentiated from other diagnoses similar in clinical presentation such as aggressive periodontitis or periodontitis as a manifestation of systematic disease (Armitage 1999). These other diseases possess different etiologies and pathogeneses. Accurate diagnosis allows formation of an appropriate treatment plan.

The presence of bacteria is widely accepted to be the etiology of chronic periodontitis. Although over 500 species of microorganisms have been identified in periodontal pockets, it is likely that only a small percentage of these are etiologic agents (Moore & Moore 1994). These select bacteria possess pathogenic characteristics, such as virulence factors which overwhelm or

subvert the host immune system, causing periodontal destruction. Additionally, host susceptibility plays a major role in whether or not the bacterial presence will result in the development of periodontitis. Genetic factors involving dysfunctional neutrophils (Van Dyke et al. 1985), IL-1 polymorphism (Karimbux et al. 2012), and hyper-responsive monocytes (Garrison & Nichols 1989) have been hypothesized to contribute to various forms of periodontitis.

The overall clinical goals of treating chronic periodontitis are to reduce gingival inflammation, arrest or slow the progression of periodontal destruction, restore the lost periodontium when possible, and bring the patient into comfortable function. This is accomplished by addressing bacteria with disruption of the biofilm, reducing microbial load, and minimizing future recolonization. Treatment objectives aim to reduce PD, gingival inflammation (BOP), plaque index (PI), and gain CAL. Treatment modalities vary and may include non-surgical and/or surgical therapies.

Non-surgical therapy consists of debridement of the teeth and involves scaling and root planing. This may include the use of hand instruments such as curettes and/or ultrasonic scalers. Instrumentation of the crown and root removes plaque and calculus, reduces subgingival bacterial load (Socransky et al. 2013), and detoxifies the roots (Nishimine & O'Leary 1979). Many studies have demonstrated that scaling and root planing improves the periodontal health in reducing PD, BOP, PI, and gaining CAL (Kaldahl et al. 1996a, Becker et al. 2001, Hung & Douglass 2002). Scaling and root planing is considered the "gold standard" of treating periodontal disease (Cobb 2002). Other factors (e.g., occlusal trauma, iatrogenic restorations, tooth crowding, smoking) that may be contributing to the disease may also be addressed in conjunction with scaling and root planing. In some cases, surgical therapy is recommended and may coincide with or may take place after non-surgical therapy to treat non-responding sites.

Long-term success of treating periodontal disease is well-documented with high tooth survival rates, CAL stability, reduced PDs, and reduced inflammation (Kaldahl et al. 1996a, Becker et al. 2001, Hung & Douglass 2002). Following active therapy (non-surgical or surgical), participation in periodontal maintenance is critical to long-term success (Nyman et al. 1975, Nyman et al. 1977, Becker et al. 1984a, Becker et al. 1984b, Wilson et al. 1987). The purpose of maintenance therapy is to disturb the subgingival bacteria and reduce the microbial load (Listgarten et al. 1978, Magnusson et al. 1984, Oosterwaal et al. 1987). This involves frequent visits (usually every 3-4 months) where the condition of the periodontium is measured and evaluated. Daily plaque control is reviewed and reinforced. Residual pockets demonstrating clinical inflammation (i.e., BOP) is indicative of histologic inflammation (Amato et al. 1986) and presence of subgingival bacteria (Wilson et al. 2008). Treatment with additional scaling and root planing is usually prescribed and is effective (Kaldahl et al. 1996b). Patients that receive active therapy and decline maintenance care usually regress back to an active diseased state (Axelsson & Lindhe 1981, Becker et al. 1984a, Becker et al. 1984b).

However, not all patients or sites respond well to conventional periodontal therapies. Reduction in bacteria after instrumentation is not always sufficient for an adequate host response (Slots et al. 1979). Consequently, adjunct therapies to scaling and root planing have been employed such as systemic antibiotics (Sgolastra et al. 2012, Garcia Canas et al. 2015), local delivery of antibiotics (Bonito et al. 2005), subgingival irrigation (Shiloah & Hovious 1993), and lasers (Cobb 2006) with mixed results. The purpose of adjunctive therapies is to further decrease the subgingival microbiota so the immune system is able to elicit a sufficient healing response. Chemical pocket disinfection is one such adjunctive therapy that has long been used without any evidence of its efficacy in the inflamed pockets of maintenance patients (Kalkwarf et al. 1982).

## LITERATURE REVIEW: CHEMICAL POCKET DISINFECTION/CHEMICAL CURETTAGE

***History and findings:***

The development of chemical pocket disinfection originated with the procedure called "gingival curettage." The stated objective of curettage is to remove the sulcular epithelium and any chronically inflamed tissues in the pocket walls, which would theoretically promote pocket shrinkage and new junctional epithelium or connective tissue attachment to the tooth.

Curettage is accomplished by using mechanical instruments, such as a curette (Hirschfeld 1952) or ultrasonic curette (Goldman 1960, Nadler 1962), along the pocket wall and is oftentimes performed in conjunction with scaling and root planing. However, gingival curettage frequently results in incomplete removal of pocket epithelium (Stone et al. 1966, Waerhaug 1955, Vieira et al. 1982), with the exception of surgical removal by incision (Yukna 1976). The use of chemical solutions, also known as "chemical curettage," was subsequently investigated to address the shortcoming of incomplete epithelium removal. Various solutions (e.g., sodium sulfide, phenol camphor, antiformin, sodium hypochlorite) have been studied for this purpose (Miller & Sorrin 1927, Waerhaug & Loe 1958); however, only studies using sodium hypochlorite were included in this review. In early literature, the generic term "antiformin" was commonly used to describe various mixtures of sodium hypochlorite solutions and can be read interchangeably with the term "sodium hypochlorite."

Chemical curettage was introduced as an aid to periodontal therapy in the early 1900s (Hecker 1913) in which solutions (e.g., sodium hypochlorite/antiformin) removed pocket epithelium via tissue necrosis. In the 1950s, a group of Canadian clinicians published descriptive techniques using chemicals to facilitate gingival curettage therapy (Box 1952, Box 1953, Shaw 1953). Their anecdotal findings claimed that chemical curettage therapy was safe, rapid, and provided predictable removal of all epithelium from the pocket. They further claimed that the

chemical removal of soft tissue was limited to epithelium, but later studies proved that its chemical action could progress further into the connective tissue (Glickman & Patur 1955, Hunter 1955, Johnson & Waerhaug 1956). Consequently, most clinicians stopped using this therapy due to its uncontrollable invasive potential. A later study by Kalkwarf et al. (1982) showed that with a strict protocol, the chemical action could be predictably limited and would heal normally. The appropriate time of chemical application was determined in this study to be one minute, followed by neutralization, and then debris removal with six curette strokes. Histological evidence of complete removal of pocket epithelium with normal healing was shown with this protocol.

The immediate effect of chemical curettage consists of complete necrosis of the epithelium and superficial layer of connective tissues forming a necrotic layer. Greater levels of inflammation lead to less uniformity of chemolytic effects and tissue necrosis (Kalkwarf et al. 1982). In a monkey study which observed histologic healing after application of sodium hypochlorite, the necrotic layer was mostly removed by the host's normal inflammatory response after 16 hours, epithelial lining had reformed after 3 days, and nearly complete healing was achieved without any sign of irreparable damage after 11 days (Johnson & Waerhaug 1956). In a human observation of healing after sodium hypochlorite delivery, the necrotic layer was removed by the host's normal inflammatory response, epithelial lining was restored after 7 days, and ongoing fibroblastic proliferation with continued maturation of connective tissue fibers was observed after 14 days (Kalkwarf et al. 1982).

Histologic studies on healing for chemical curettage are limited; therefore, the following comments are from studies observing mechanical curettage which will be used to describe the remainder of the healing considering the great similarity of therapy. Healing is initiated by the formation of a blood clot in the pocket immediately after curettage. Dilated blood vessels are

present in the tissues and numerous neutrophils migrate to the wound surface. Granulation tissue rapidly proliferates. Neutrophil numbers decrease after 2-5 days unless bacterial plaques are present and lymphocytes and plasma cells appear. Reformation and epithelialization of the sulcus occurs in 2 to 7 days. Junctional epithelium restoration occurs in as little as 5 days. Immature collagen fibers appear within 21 days with a decrease in the number of small blood vessels as the granulation tissue matures (Moskow 1964, Stone et al. 1966).

Although healing after chemical and mechanical curettage therapies appear to be innocuous, does its healing fulfill the objective of promoting new connective tissue attachment? Other studies observed the histologic healing of gingival curettage and found no new connective tissue attachment, thus negating the main objective of removing inflamed epithelium to replace with connective tissue attachment. In a beagle dog study, scaling and root planing with sodium hypochlorite-citric acid solutions were applied to ligature-induced periodontal pockets and compared to scaling and root planing with mechanical soft tissue curettement. No difference in healing between mechanical or chemical curettage was observed, which was by long junctional epithelium (Vieira et al. 1982). Similar healing was seen in Rhesus monkey studies that employed scaling and root planing with mechanical curettage (Caton & Zander 1979, Caton et al. 1980) and in another Rhesus monkey study with complete epithelium removal by surgical incisions (Yukna 1976). “Windows” of connective tissue attachment interrupting the long junctional epithelium was noted in one of these studies (Caton & Zander 1979). In a study employing subgingival plaque removal without any intentional curettage, a similar long junctional epithelium formed (Waerhaug 1978). It is generally accepted that curettage heals with a long junctional epithelium similar to healing accomplished from scaling and root planing.

Chemical curettage has been shown to eliminate bacterial loads in pockets. A study by Adcock et al. (1983) showed that chemical curettage does indeed have bactericidal effects and

can effectively eliminate bacteria from deep periodontal pockets. Sodium hypochlorite solution was applied to the periodontal pockets of patients less than 18 years of age with aggressive periodontitis, without any scaling and root planing. The results of the study were solely attributed to the bactericidal effects of the solution. The findings observed a significant decrease in the number of gram-negative anaerobes and spirochetes that lasted for 30-90 days.

Scaling and root planing alone has also been shown to reduce bacterial loads in pockets by 10- to 100-fold. Gram-negative anaerobes and spirochetes were significantly reduced and showed a 1-6 month duration until these microorganisms repopulated to baseline levels (Slots et al. 1979). One would logically deduce that a combination of scaling and root planing with adjunctive chemical curettage would yield improved results microbiologically and even clinically; however, a study by Forgas & Gound (1987) showed otherwise. Scaling and root planing alone was compared to scaling and root planing with adjunctive chemical curettage using sodium hypochlorite. The percentages of spirochetes and motile rods in subgingival plaques were observed. Similar reductions were observed after post-therapy accompanied by gradual returns to baseline levels after 12 weeks. There was no difference at any time between therapies.

Not all patients respond to scaling and root planing with a reduction in periodontal pathogens. Antibiotics may be necessary to enhance the disturbance of the flora in the subgingival plaques of periodontal pockets. Two out of six patients in a study observing the microbial response to scaling and root planing resulted in an insignificant shift of flora after two rounds of mechanical instrumentation. Only after tetracycline was administered was a significant flora shift and reduction achieved (Slots et al. 1979). Chemical curettage may enhance the antimicrobial reduction in patients with sites not responding to conventional periodontal therapy and should be investigated.

Another benefit may be a slight improved visibility and access gained from the removal of pocket soft tissue resulting in improved mechanical removal of plaque, calculus, and biofilm. This benefit has not been studied.

No studies had evaluated the beneficial clinical effects of chemical curettage and directly compared it to a control group until Forgas & Gound (1987) compared scaling and root planing alone to scaling and root planing with adjunctive chemical curettage using sodium hypochlorite and citric acid. Periodontal measures were recorded and no difference in PD reduction or CAL gain was found between groups leading to the conclusion that chemical curettage did not provide additional benefits to scaling and root planing.

The history of curettage has been controversial. Studies on gingival or chemical curettage show no healing or clinical benefits in treating periodontitis. Upon review of the literature on gingival curettage, the American Academy of Periodontology released a statement in 2002 stating that gingival curettage "has no additional benefit to SRP [scaling and root planing] alone in the treatment of chronic periodontitis" (AAP Statement 2002). Resultantly, most research on sodium hypochlorite for curettage use in periodontal therapy has halted. Most dentists no longer use chemical curettage in initial periodontal therapy today, but some still implement it in their periodontal maintenance patients. No studies have evaluated its potential role in periodontal maintenance as a pocket disinfectant to reduce inflammation in select sites.

***Sodium hypochlorite properties and mechanism of action:***

Sodium hypochlorite has been used as a disinfectant for more than 100 years, as an antiseptic for more than 85 years, and as an endodontic irrigant for more than 75 years. It has many of the properties of an ideal antimicrobial agent due to its high pH, including broad antimicrobial activity, rapid bactericidal action, no color, no staining, ease of access, and very low cost. The active species is undissociated hypochlorous acid and is lethal to most bacteria,

fungi, viruses, as well as any other organic tissues. Its mechanism of action is reduced by the presence of organic material, heavy metal ions, and low pH (Slots 2002).

Sodium hypochlorite inhibits key enzymatic reactions within the cell, denatures protein, and inactivates nucleic acids. It interacts with infectious organisms and host cells through three main reactions: saponification, neutralization, and chloramination. When sodium hypochlorite contacts fatty acids, a saponification reaction occurs yielding soap (fatty acid salt) and glycerol (alcohol). When it contacts amino acids, either a neutralization or chloramination reaction occurs yielding water and salt, or chloramine and water, respectively (Estrela et al. 2002).

Highly concentrated sodium hypochlorite is a strong base (pH > 11). The high pH alters the integrity of the cytoplasmic membrane by denaturing proteins and phospholipids of the membrane. It also irreversibly inhibits enzymes within the membrane. Sodium hypochlorite in high concentrations will more aggressively degrade microbes and organic tissue while lower concentrations (0.5-1%) are biocompatible (Estrela et al. 2002). Sodium hypochlorite-specific resistance by bacteria has yet to be reported (McDonnell & Russell 1999).

***Other Dental Uses:***

Sodium hypochlorite is primarily used in dentistry today as an endodontic irrigant and is commonly used at a concentration of 5.25%. It first appeared in the endodontic literature in 1920 (Crane 1920) and is now routinely used around the world. Sodium hypochlorite is an effective antimicrobial and has tissue-dissolving capabilities. Its benefits include having a low viscosity (allowing easy introduction into the canal architecture), an acceptable shelf life, wide availability, and low cost. The main disadvantages in dental use are the toxicity to vital tissues and corrosion of metals (O'Hoy et al. 2003). It is possible to possess an allergy to sodium hypochlorite, although a few clinical reports indicate it is very rare (Caliskan et al. 1994).

Sodium hypochlorite is also used as a solution in subgingival irrigation and mouth rinse. In subgingival irrigation, a dilute solution of 0.5% sodium hypochlorite is expressed directly into the pocket during periodontal therapy with the objectives of reducing microbial load and plaque pH. Using diluted doses of sodium hypochlorite in subgingival irrigation, although inherently different from the chemical curettage protocol, has been shown to reduce plaque and gingivitis, as well as reduce plaque pH levels for 24 hours even with the challenge of a sugary rinse (Lobene et al. 1972). The American Dental Association Council on Dental Therapeutics has designated dilute sodium hypochlorite as a “mild antiseptics mouth rinse” and suggested its use for direct application to mucous membranes and wounds (ADA 1984).

**LITERATURE REVIEW: PERIODONTITIS, INTERLEUKEN-1β, AND INTERLEUKIN-1ra**

Periodontal disease is caused by bacteria in dental plaque with evidence that specific bacterial pathogens are responsible for the progressive form of the disease. However, some individuals possess these specific microorganisms but do not appear to show evidence of disease progression (Haffajee et al. 2004). This implies that there are various degrees of patient susceptibility which may involve the host immune system. Although periodontal bacteria are the major etiological agents, the host immune response to these bacteria is of fundamental importance.

Detecting susceptible individuals is currently difficult. There is great variability in the microbial composition between individuals and from site to site in the same individual. Each bacterium has a unique set of virulence factors and strains with various phenotypes (Griffen et al. 1999). Most periodontal disease is chronic; however, the nature of its chronicity is not entirely known with respect to the frequency and rate of disease progression. It is hypothesized that periodontitis is either a continuous or episodic process, or a combination of the two (Goodson et al. 1982, Gilthorpe et al. 2003). There are still many aspects of periodontal disease not yet understood.

What is known is that there is an established relationship between periodontal destruction and inflammation (Van Dyke 2008). The interaction between periodontal pathogens and immune system results in chronic gingival inflammation; thus, leading to progressive destruction of nearby connective tissue attachment and alveolar bone around the teeth. In the acute phase of the periodontal lesion, large numbers of neutrophils migrate toward the infected site. In the latter phase, a dense infiltrate forms composed mainly of lymphocytes, macrophages, and plasma cells. As the lesion becomes more established, the loss of collagen and fibroblast alteration increases (Page & Schroeder 1976). Periodontal destruction is

dependent on the concentration of inflammatory mediators present in gingival tissue and penetration of these mediators within the gingival tissue to reach a critical distance from the alveolar bone (Graves & Cochran 2003). Page & Schroeder (1981) showed that bone resorption ceases when a 2.5 mm zone is created between the site of bacteria and bone. They concluded that the closer inflammatory cells are to the bone, the greater the amount of degradation.

Inflammation involves both the innate and adaptive immune responses (Graves 2008). Leukocytes are recruited within the innate and adaptive responses and are the primary producers of cytokines that initiate and sustain inflammation. Cytokines are cell signaling molecules that aid cell-to-cell communication in immune responses. Cytokines stimulate the movement of cells toward sites of inflammation and infection, and influence the production and activation of different effector cells. A delicate balance of cytokine regulation is necessary for disease to be controlled. Heightened cytokine production may result in more destructive or progressive disease. Further study of cytokines may shed light on the host response on why some individuals may be more susceptible to periodontal disease than others and how to better control inflammatory disease.

It is possible to measure cytokines in GCF of diseased pockets to study inflammation with the use of enzyme-linked immunosorbent assay (ELISA) techniques. GCF is an inflammatory exudate originating from subgingival microvasculature that can be collected within the gingival sulcus. Although GCF originates from the vasculature, systemic cytokine levels of the blood serum do not accurately depict the inflammatory state in the periodontium, or vice versa, as most of the cytokines are released locally and not systemically in periodontal disease (Orozco et al. 2006, Trombelli et al. 2010). Several inflammatory markers have been identified in GCF of periodontally involved teeth, including interleukin-1 beta (IL-1β) (Masada et al. 1990, Preiss &

Meyle 1994) and interleukin-1 receptor antagonist (IL-1ra) (Kabashima et al. 1996, Ishihara et al. 1997).

IL-1β is a pro-inflammatory cytokine that is released upon activation of the host inflammatory response to bacteria (Ishihara et al. 1997, Cochran 2008). It is a glucoprotein of 17 kDa and binds to IL-1 receptor (Dinarello 1991). IL-1β is produced predominantly by monocytes and macrophages (Mergenhagen 1984, Matsuki et al. 1992), and also by fibroblasts, dendritic cells, Langerhans cells, B cell lines, endothelial cells, neutrophils, epithelial cells, and bone cells (Oppenheim et al. 1986, Horowitz 1993). Its production may be induced by microorganisms, microbial products, inflammatory agents, and antigens (Preiss & Meyle 1994). Some of its pro-inflammatory effects include: stimulation of T-lymphocytes and lymphokine production (Mizel 1987), proliferation of B-lymphocytes and antibody production (Chiplunkar et al. 1986), fibroblast proliferation, stimulation of prostaglandin ($PGE_2$) released by monocytes and fibroblasts, enhancement of neutrophil chemotaxis and activation (Sauder et al. 1984), and release of metalloproteinases that degrade extracellular matrix proteins (Dinarello 1991). Critical to the prognosis of periodontal disease, IL-1β also promotes osteoclast formation and is a potent inducer of bone demineralization (Dewhirst et al. 1985). IL-1β is a major mediator of tissue destruction in periodontal disease (Page et al. 1997).

IL-1β can be predictably measured in GCF and has been shown to be present at an increased level in periodontally involved sites compared to healthy sites. A study by Preiss & Meyle (1994) sampled diseased sites from 19 untreated patients with moderate to severe periodontitis and from 14 sites in healthy control patients. All samples successfully detected IL-1β. The diseased patients had a mean concentration of 313 ng/mL (range: 132-844) and healthy patients had a mean of 73 ng/mL (range: 35-141). Another study by Goutoudi et al. (2004) recruited 12 patients with moderate to advanced levels of periodontal disease and sampled 24

non-diseased sites and 72 diseased sites multiple times. IL-1β was successfully detected in 382 out of 384 samples and the mean total amount of IL-1β was significantly higher in diseased sites compared to non-diseased sites at baseline.

The amount of IL-1β in GCF is closely associated with the severity of periodontal disease and active inflammation. Ishihara et al. (1997) evaluated the correlation of IL-1β levels in GCF and the clinical status of patients with slight, moderate, or severe levels of periodontitis and healthy controls. No IL-1β was detected in the GCF obtained from non-inflamed sites of the healthy subjects. The total amount of IL-1β was correlated with alveolar bone loss. Engebretson et al. (2002) showed a strong correlation with IL-1β levels and both pocket depth and attachment levels. Conversely, Masada et al. (1990) found no correlation of IL-1 (α and β) levels and pocket depth. This was explained by pocket depth being only reflective of cumulative history of periodontal disease and does not indicate current disease activity. In a similar study, Mogi et al. (1999) measured IL-1β and found an association with levels of IL-1β and pocket depth and BOP (active inflammation). It is generally concluded that measuring the IL-1 biomarker in GCF may be valuable in detecting the activity of inflammation and breakdown in periodontal tissues.

In the initial and maintenance phase of periodontal therapy, pockets with inflammation (i.e., BOP) are primary targets due to the likely presence of root surface irritants (Ramfjord 1987, Ramfjord et al. 1987). Reinhardt et al. (2010) investigated the relationship between IL-1β and subsequent attachment and bone loss in postmenopausal women with moderate to advanced periodontitis. Maintenance patients who experienced an increase in IL-1β from the previous year's visit were twice as likely to have progression of periodontitis in the following year.

Scaling and root planing has been consistently shown to reduce clinical inflammation and the amount of IL-1β in periodontally diseased sites (Hou et al. 1995, Tsai et al. 1995,

Engebretson et al. 2002, Goutoudi et al. 2004). This reduction in IL-1β may last up to 24 weeks before returning to baseline levels (Engebretson et al. 2002). Smoking may have a negative effect on periodontal therapy as shown by Goutoudi et al. (2004). At baseline, smokers and non-smokers had similar IL-1β levels. After therapy, smokers had significantly higher levels of IL-1β than non-smokers. Non-steroidal anti-inflammatory drug (NSAID) intake was not shown to significantly influence IL-1β in GCF (Bostrom et al. 2000, Kim et al. 2007, Buduneli et al. 2010).

IL-1ra belongs to the IL-1 family and binds to IL-1 receptors with nearly the same avidity as IL-1β (Arend 2002). Its binding to IL-1 receptors does not induce a cellular response, thereby antagonizing the effects of IL-1β (Seckinger et al. 1987, Dinarello 2013). It is a 17-23 kDa protein secreted by immune cells (Roux-Lombard et al. 1989, McColl et al. 1992, Hagaman et al. 2001), epithelial cells (Perrier et al. 2002), keratinocytes (Gruaz-Chatellard et al. 1991), stromal cells (Chan et al. 1992, Kristensen et al. 1992), and adipocytes (Juge-Aubry et al. 2004). An excess of IL-1ra up to at least 100-fold is necessary to counteract the effects of IL-1 in vitro (Arend et al. 1990). The function of IL-1ra is regulated only by its levels of production, since it does not induce signal transduction (Perrier et al. 2006).

IL-1ra plays a defensive role in periodontitis; however, an increased secretion of IL-1ra is insufficient to overwhelm the release of IL-1β. Gilowski et al. (2014) showed that IL-1ra was significantly higher (over 1.5x greater in mean moles/sample) in the periodontitis group than in the control group. Holmlund et al. (2004) also showed significantly higher levels of IL-1ra in diseased versus control groups. Rawlinson et al. (2000) and Toker et al. (2008) showed contrasting results with the diseased groups having significantly lower levels of IL-1ra than the control group.

Ishihara et al. (1997) showed no correlation with IL-1ra and alveolar bone loss scores while Toker et al. (2008) showed a negative correlation with IL-1ra and gingival index in

moderate pockets only. Evaluating the response to periodontal therapy, one would intuitively think that IL-1ra would increase to nullify the effects of IL-1β, since clinical inflammation is often reduced. However, Toker et al. (2008) showed that IL-1ra did not significantly change at diseased sites after scaling and root planing. After surgical therapy of sites with osseous defects, Holmlund et al. (2004) showed a non-significant decrease in IL-1ra.

To better understand the role IL-1ra plays in periodontitis, IL-1ra cannot be studied alone, but rather the relationship of IL-1ra to IL-1β needs to be investigated. An imbalance between IL-1β and IL-1ra is one of the factors influencing the course, susceptibility, and severity of many diseases other than periodontitis including kidney/liver/pancreas/central nervous system diseases, arthritis, inflammatory bowel disease, granulomatous and fibrotic lung disorders, graft-versus-host disease, leukemia and cancer, osteoporosis, diabetes, infectious diseases, and arterial diseases (Arend 2002). Either an overproduction of IL-1β or underproduction of IL-1ra predisposes individuals to these diseases. The increase in circulating IL-1ra levels corresponds to a delayed event in response to IL-1β production and may represent a preventive mechanism in chronic inflammation (Opp et al. 1992). A large randomized controlled trial evaluated the efficacy of IL-1ra therapy in 472 patients with active and severe rheumatoid arthritis and found beneficial effects on the rate of joint erosion (Bresnihan et al. 1998, Bresnihan et al. 2004). Increasing the ratio of IL-1ra/IL-1β may be therapeutic in treating periodontal disease. GCF concentration of IL-1ra was approximately 1000-fold that of IL-1β in periodontitis patients (Bostrom et al. 2000). Ishihara et al. (1997) reported that patients with slight alveolar bone loss had pre-therapy IL-1ra moles 600-fold that of IL-1β and severe bone loss patients only a 90-fold. Gilowski et al. (2014) reported GCF molar levels of IL-1ra to be 800-fold that of IL-1β in the control group and only 300-fold in the periodontitis group, indicating a decrease in IL-1ra/IL-1β ratio. Further study of the IL-1ra/IL-1β ratio may be helpful in better

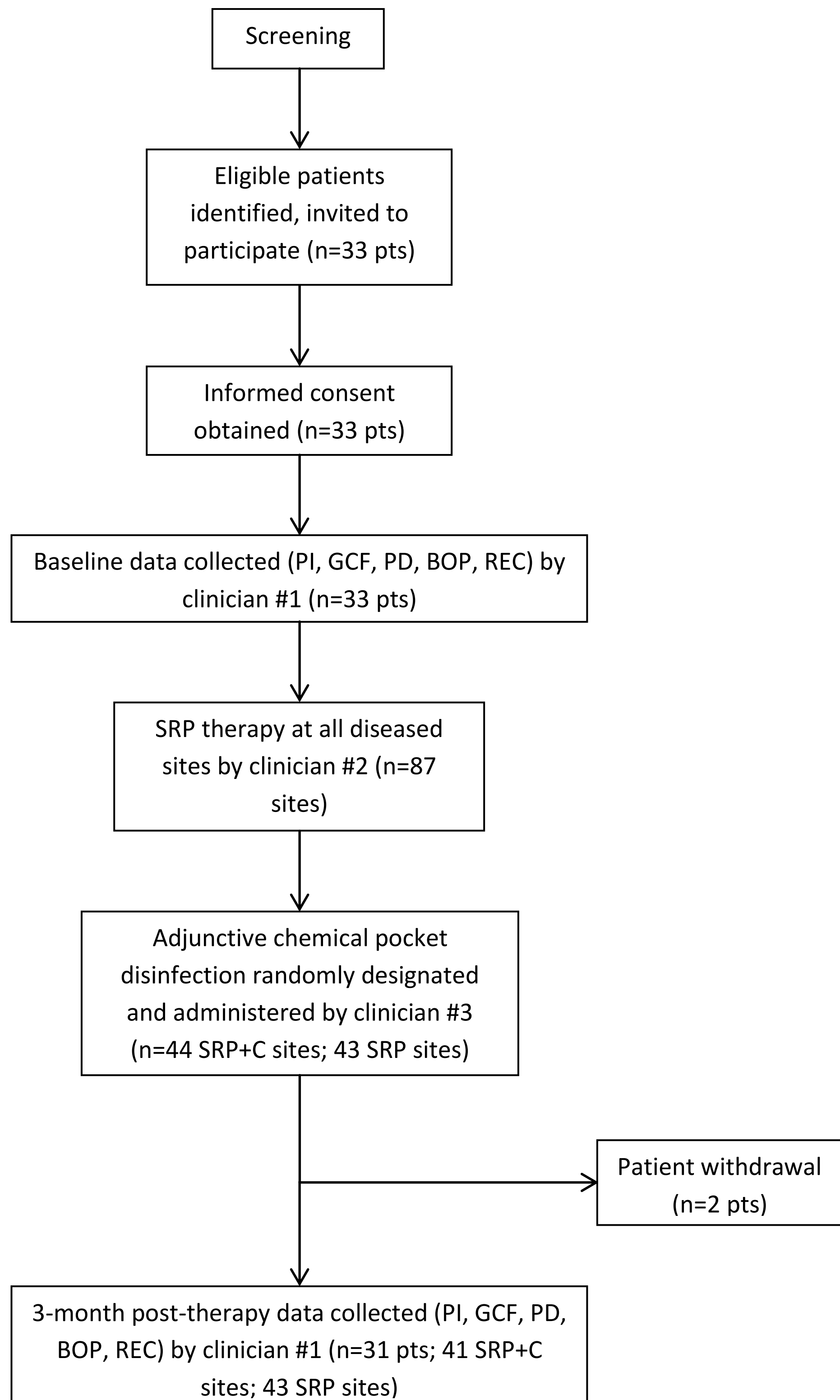
understanding the dynamics of this delicate balance of anti- and pro-inflammatory cytokines, and their role in periodontitis etiology and therapy.

## MATERIALS AND METHODS

### ***Overview of study design:***

Eligible patients with qualifying diseased and healthy sites were identified. An invitation to participate in the study was extended and consent obtained. Clinical data and GCF samples were collected from each study participant at sites of interest by a calibrated, blinded clinician. All diseased sites (test and control) received scaling and root planing by a second clinician not knowing which sites would receive the subsequent chemical pocket disinfection. A third clinician then randomly assigned which diseased sites would receive adjunctive chemical pocket disinfection (test) and administered this therapy. Data collection and therapies were performed at the same visit. Study participants returned 3-months (± 2 weeks) post-therapy for final data collection by the initial, blinded clinician.

**Figure 1:** Flow of study design



***Patient selection:***

The clinical phase of the study was conducted from February 2014 to November 2014. Patients receiving regular periodontal maintenance therapy at the University of Nebraska Medical Center College of Dentistry were screened by a staff dental hygienist (MC) at their scheduled maintenance appointment. The inclusion criteria consisted of any adult periodontal maintenance patient (≥ 30 years of age) with a history of therapy for chronic periodontitis, whose last maintenance therapy occurred within the past 3-6 months, and had one or more sites of ≥ 5 mm PD with BOP. Non-smokers and smokers were included in the study. Exclusion criteria consisted of subjects who were uncontrolled diabetics, pregnant, used NSAIDs or anticoagulants regularly for chronic disease/pain, used antibiotics in the previous three months, or required antibiotic coverage for dental treatments. Patients that met the inclusion criteria were invited to participate in the study. Each screened patient interested in participating in the study had their diseased sites and history confirmed by a trained dentist (CR). The study protocol was explained, questions answered, and consent obtained from all subjects. Maintenance therapy was performed at that time by MC, leaving the sites of interest untreated. The patient returned as soon as possible for clinical data collection followed by administration of the assigned therapy. Study participants would return in three months for post-treatment data collection immediately followed by resumption of their regular maintenance therapy, thus minimally interrupting ongoing periodontal therapy. The experimental protocol was approved by the University of Nebraska Medical Center Institutional Review Board (IRB# 636-13-FB) and registered at ClinicalTrials.gov (ID# NCT02316652).

***Data collection:***

Data collection was composed of two parts: clinical data and GCF collection. Clinical data were collected from diseased study sites (pockets ≥ 5 mm with BOP), adjacent sites, and one healthy (H) site (PD ≤ 4 mm with no BOP) from each patient at baseline and 3-months post-therapy. The clinical data were collected at six sites per tooth (mesio-, mid-, and disto-facial; mesio-, mid-, and disto-lingual) of the study teeth and adjacent teeth. GCF collection was performed at the diseased study sites and one H site from each patient.

First, supragingival plaque was scored by passing a periodontal probe tip with a single pass along the tooth surface at the free gingival margin. Plaque was recorded using Silness & Loe's PI (see Appendix A).

Second, GCF samples were collected. Any residual supragingival plaque was cleared using an explorer or curette. The site was gently dried using gauze and/or air syringe, and isolation established using cotton rolls. GCF was collected by placing a sterile paper absorptive strip (PerioPaper strips, Oraflow Inc., Smithtown, NY) into the pocket or sulcus until slight resistance was detected. The strip was removed after 30 seconds and immediately placed in sterile Eppendorf micro-test tubes which were then stored in a secure freezer kept at -80°C until further analysis. GCF strips contaminated with blood were discarded and collection was repeated.

Third, the remaining clinical parameters including PD, relative REC, and BOP were measured. PD was measured as the distance from the free gingival margin to the base of the pocket or sulcus using a periodontal probe (UNC-15 probe, Hu-Friedy, Chicago, IL) in the deepest point of each site. REC was defined as the measured distance from the cemento-enamel junction or restorative margin to the free gingival margin at the measured location of each PD. The presence of BOP was scored as present or absent (+/-) within 30 seconds from the time of

probing. Relative CAL was calculated as the sum of the PD and REC measurements. All clinical measures and GCF collection were performed by one calibrated, blinded examiner (CR).

***Treatment protocol:***

All identified study and adjacent sites were treated with scaling and root planing by a single clinician (MB) not involved with clinical measurements or future random allocation of sites to receive subsequent chemical pocket disinfection. Scaling and root planing was performed utilizing hand and ultrasonic instruments until a smooth, glassy root surface was obtained at the discretion of the clinician (MB). No local anesthesia was utilized.

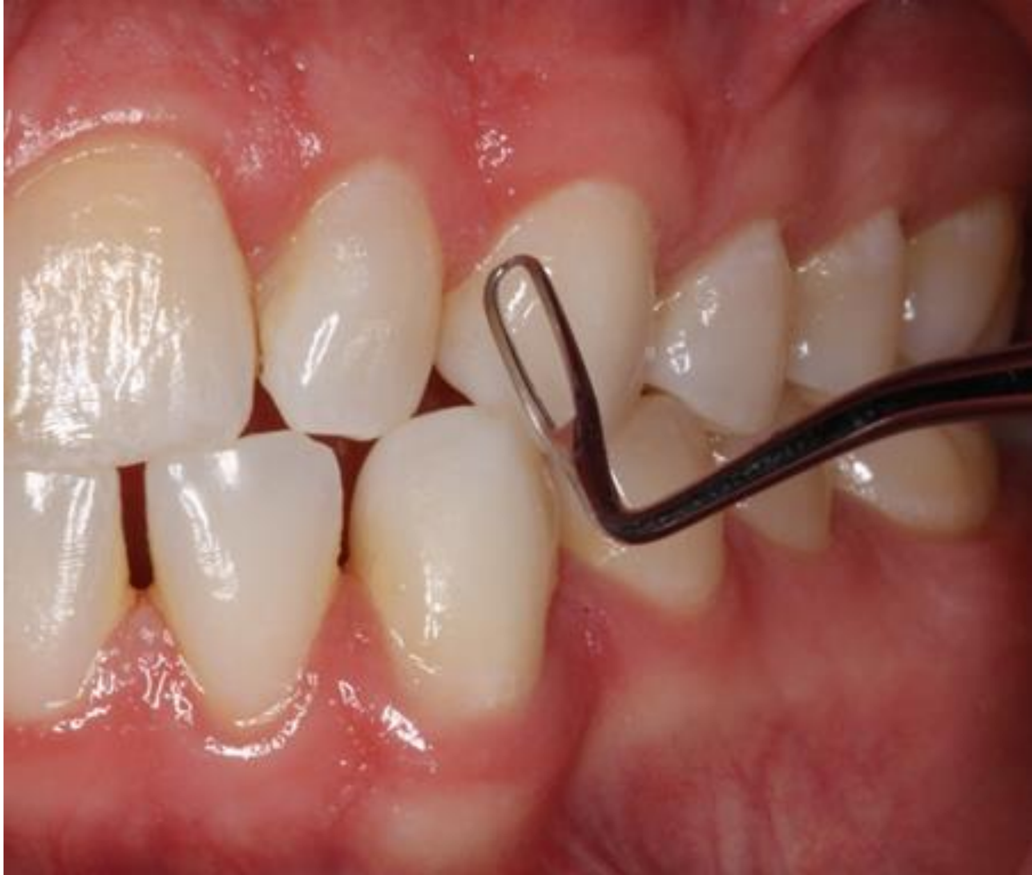
Following instrumentation, another clinician (WK) randomly allocated which study sites would receive chemical pocket disinfection therapy. Randomization was decided by the flip of a coin at every other site in numerical order, thereby alternating therapy assignment equally. Sites receiving chemical therapy (test) could not be in the same quadrant as sites not receiving this therapy (control) to prevent any chance of communication or influence due to close proximity. Multiple sites in the same quadrant receiving the same therapy were acceptable. Adjacent sites were also treated with chemical therapy. Plaque control was reinforced.

Chemical pocket disinfection therapy included cotton roll isolation of the area to be treated. A 6% sodium hypochlorite solution (Wagey Drug Co., Lincoln, NE) (see Appendix B) was inserted into the pocket using a modified Orban medicament loop (Hu-Friedy, Chicago, IL) (see Figure 1). After 60 seconds, a 5% neutralizing citric acid solution (Wagey Drug Co., Lincoln, NE) was inserted into the pocket using the medicament loop. The site was then irrigated with water and debris removed with a curette.

The sodium hypochlorite solution was verified to have a pH of 14. The sodium hypochlorite and citric acid solutions were stored in a cool, dark environment.

At the conclusion of treatment, diseased sites received either test therapy: scaling and root planing with adjunctive chemical pocket disinfection (SRP+C), or control therapy: scaling and root planing alone (SRP).

**Figure 2:** Modified Orban medicament loop used to deliver chemical solutions



***Analysis of GCF samples:***

GCF samples were collected at all diseased sites and one H site per patient. Only GCF samples from paired, treated sites (i.e., one test and one control within each patient), plus the H site sample, were analyzed.

GCF samples were analyzed for IL-1β and IL-1ra using ELISA based on a quantitative sandwich technique. All assay procedures were performed by individuals (CR, MS) without knowledge of the therapy allocation and according to the manufacturer's protocol using human recombinant standards. The stored GCF samples were allowed to thaw at room temperature. The sample strips were eluted in 1 mL of phosphate buffer solution and gently agitated for 1 hour.

IL-1β:

Two-hundred µL of each standard and reconstituted GCF sample were aliquoted in duplicate to wells pre-coated with monoclonal antibody specific for IL-1β (Human IL-1β/IL-1F2 Quantikine® ELISA, R&D Systems, Minneapolis, MN). After 2 hours of incubation at room temperature, the wells were aspirated and washed 3 times. Two-hundred µL of human IL-1β conjugate were added to each well and incubated for 2 hours at room temperature. Aspiration and washes were again performed 3 times. Two-hundred µL of substrate solution were added to each well and incubated for 20 minutes at room temperature in the absence of light. Fifty µL of stop solution were added to each well and gently agitated until a yellow color was obtained. The microplate was read at a wavelength of 450 nm and corrected for optical imperfections by subtracting the 570 nm readings to obtain the optical density of each well.

The IL-1β standard calibration curves were generated. The minimum detectable concentration was 0.878 pg/mL and the maximum detectable concentration was 262 pg/mL.

Each GCF sample was analyzed separately. The optical density and relative IL-1β concentration of each sample were estimated from the standard curve. Cytokine values lower than the lowest detectable level were set to the lowest detected level on the microplate's standard curve (occurred in one H site). Cytokine values higher than the maximum detectable level were set to 262 pg/mL (occurred in 13 diseased sites).

The IL-1β concentration of a GCF sample from each site was the average of each sample's duplicate. The concentration (pg/mL) was multiplied by the volume of the sample used for the ELISA (x0.2 mL), then multiplied by the proportion of used sample (x5) to calculate the total IL-1β amount per 30-second sample.

IL-1ra:

Due to the high quantities and large range of IL-1ra present in GCF, dilutions of 1:10 and 1:100 of the original eluted sample were made to fit in the standard curve. One-hundred µL of each standard and reconstituted GCF sample were aliquoted in duplicate to wells pre-coated with monoclonal antibody specific for IL-1ra (Human IL-1ra/IL-1F3 Quantikine® ELISA, R&D Systems, Minneapolis, MN). After 2 hours of incubation at room temperature, the wells were aspirated and washed 4 times. Two-hundred µL of human IL-1ra conjugate were added to each well and incubated for 2 hours at room temperature. Aspiration and washes were again performed 4 times. Two-hundred µL of substrate solution were added to each well and incubated for 30 minutes at room temperature in the absence of light. Fifty µL of stop solution were added to each well and gently agitated until a yellow color was obtained. The microplate was read at a wavelength of 450 nm and corrected for optical imperfections by subtracting the 570 nm readings to obtain the optical density of each well.

The IL-1ra standard calibration curves were generated. The minimum detectable concentration was 6.3 pg/mL. The maximum detectable concentration was 2,100 pg/mL.

Each GCF sample was analyzed separately. The optical density and relative IL-1ra concentration of each sample were estimated from the standard curve. No cytokine values were lower or higher than the minimum or maximum detectable levels.

The IL-1ra concentration of a GCF sample from each site was the average of each sample's duplicate. The IL-1ra concentration measured from the 1:10 dilution was used unless it was greater than the maximum detectable level. If IL-1ra levels were greater than the maximum detectable level in the 1:10 dilution, the values measured in the 1:100 dilution microplates were then utilized. When using the appropriate dilution, no cytokine values were outside the standard curve. A dilution factor of 10 was used in the 1:10 dilution samples and a dilution factor of 100 was used in the 1:100 dilution samples to calculate the appropriate IL-1ra concentrations. The concentration (pg/mL) was multiplied by the volume of the sample used for the ELISA (x0.1 mL), then multiplied by the proportion of used sample (x10) to calculate the total IL-1ra amount per 30-second sample.

Anti-inflammatory index (IL-1ra/IL-1β ratio):

IL-1ra amounts per 30-second sample were divided by IL-1β amounts per 30-second sample to obtain IL-1ra/IL-1β ratios. This allowed a positive whole number for the ratio and could be considered an "anti-inflammatory index." This ratio also has been used in the medical literature (Casini-Raggi et al. 1995, Carter et al. 2004, Richette et al. 2008).

***Statistical analyses:***

The ideal sample size to ensure adequate power was calculated to ensure detection of 0.5 mm change in PD from baseline to 3-months post-treatment between therapies. It was determined that 42 treatment sites per group would be necessary to provide an 80% power with $\alpha$=0.05.

Intra-therapy analyses were performed to identify the changes in PD, CAL, BOP, and PI between baseline and 3-months post-treatment within each therapy. Inter-therapy analyses were performed to compare the differences between therapies at baseline and 3-months post-treatment for PD, CAL, BOP, PI, IL-1β, IL-1ra, and the anti-inflammatory index (IL-1ra/IL-1β ratio).

Data were analyzed using a paired t-test and a mixed model with the patient as the random effect and therapy as the fixed effect. Chi-square and the binomial procedure analyses were used to evaluate BOP. Analyses with *P* values less than 0.05 were considered statistically significant.

Upon analyses of the data, a difference that appeared to be significant was observed in sites where BOP resolved compared to bleeding sites that did not resolve. Further investigation was performed and the results were deemed significant enough to be reported. The report can be found in the "Outcomes based on BOP resolution" subsection (p. 33).

## RESULTS

***Examiner calibration:***

The data collection examiner (CR) was calibrated for intra-examiner reliability and reproducibility on 23 patients using 105 randomly chosen sites. PD was reproducible at ± 1 mm for 99.0% of the measures and CAL at 88.9%.

***Patient characteristics:***

All 33 patients that were eligible and invited to participate in the study agreed to do so. Two patients were unable to complete the study (6.1% dropout rate). One patient did not return for the 3-month post-treatment exam due to a cardiovascular accident resulting in hospitalization one week before the scheduled appointment. The other patient returned for the 3-month post-treatment exam but was disqualified due to an acute back injury and subsequent chronic usage of NSAIDs (one of the exclusion criterion). Both reasons for patient dropout were not believed to be related to any dental therapy. Thirty-one patients completed the study.

All study patients were asked at the 3-month post-therapy exam if any symptoms or problems were experienced. No post-operative complications were encountered or reported throughout the study.

Patient characteristics at the baseline examination are displayed in Tables 1 and 2.

**Table 1:** SRP+C patient characteristics at baseline

| | |
|---|---|
| **Total # of study patients** | 23 |
| **Mean age (range)** | 64.7 years (48-79) |
| **Female (%)** | 12 (52.2) |
| **Male (%)** | 11 (47.8) |
| **Smokers (%)** | 5 (21.7) |
| **Non-smokers (%)** | 18 (78.3) |
| **Total # of sites** | 41 |

**Table 2:** SRP patient characteristics at baseline

| | |
|---|---|
| **Total # of study patients** | 25 |
| **Mean age (range)** | 65.0 years (48-84) |
| **Female (%)** | 11 (44.0) |
| **Male (%)** | 14 (56.0) |
| **Smokers (%)** | 4 (16.0) |
| **Non-smokers (%)** | 21 (84.0) |
| **Total # of sites** | 43 |

***Clinical outcomes:***

The mean baseline and post-treatment measurements of clinical parameters and respective changes are presented in Table 3.

**Table 3:** Clinical outcomes

| Clinical Parameter | Therapy | N | Baseline (SEM) | Mean Change (SEM) | 3-mo Change *P* Value |
|---|---|---|---|---|---|
| **PD (mm)** | SRP+C | 41 | 5.90 (0.14) | -0.63 (0.14) | <0.001 |
| | SRP | 43 | 5.84 (0.14) | -0.60 (0.14) | <0.001 |
| **CAL (mm)** | SRP+C | 41 | 5.08 (0.36) | 0.44 (0.17) | <0.01 |
| | SRP | 43 | 4.84 (0.35) | 0.26 (0.16) | NS (0.18) |
| **BOP (%)**** | SRP+C | 41 | 100 | -48.8 | <0.0001 |
| | SRP | 43 | 100 | -30.2 | <0.0001 |
| **PI** | SRP+C | 41 | 1.56 (0.12) | 0.004 (0.12) | NS (1.0) |
| | SRP | 43 | 1.59 (0.12) | 0.07 (0.12) | NS (0.52) |

No statistically significant difference between SRP+C and SRP for each clinical parameter
**No SEM due to use of Chi-square analysis
NS: Not statistically significant ($P \geq 0.05$)

The mean baseline PD, CAL, BOP, and PI for SRP+C and SRP sites were not statistically different. The mean reductions in PD for both SRP+C and SRP were significant ($P < 0.001$). The

mean gain in CAL was significant only for SRP+C ($P < 0.01$). All sites initially had BOP as part of the inclusion criteria and the decrease in BOP for both SRP+C and SRP was significant ($P < 0.0001$). The difference in changes between SRP+C and SRP in PD reduction ($P = 0.88$), CAL gain ($P = 0.43$), BOP reduction ($P = 0.08$), and PI change ($P = 0.70$) were not significant.

***Cytokine outcomes:***

GCF samples from 16 patients were used. One patient's GCF samples were discarded due to collection error and were not included in the results.

Cytokine comparisons for SRP+C, SRP, and H sites are shown in Tables 4-6.

**Table 4:** IL-1β levels

| Therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **SRP+C** | 16 | 148 (16) pg[a] | 102 (15) pg[a] |
| **SRP** | 16 | 119 (16) pg[a] | 96 (15) pg[a] |
| **H** | 16 | 20 (19) pg[b] | 18 (17) pg[b] |

Dissimilar superscripts within each column indicate significant difference ($P < 0.05$)

**Table 5:** IL-1ra levels

| Therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **SRP+C** | 16 | 19,703 (2,797) pg[a] | 22,207 (2,683) pg[a] |
| **SRP** | 16 | 18,561 (2,797) pg[a] | 14,564 (2,683) pg[b] |
| **H** | 16 | 10,876 (3,124) pg[b] | 9,079 (3,014) pg[b] |

Dissimilar superscripts within each column indicate significant difference ($P < 0.05$)

**Table 6:** Anti-inflammatory index (IL-1ra/IL-1β ratio)

| Therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **SRP+C** | 16 | 345 (207)[a] | 290 (203)[a] |
| **SRP** | 16 | 284 (207)[a] | 195 (203)[a] |
| **H** | 16 | 1,365 (252)[b] | 1,477 (253)[b] |

Dissimilar superscripts within each column indicate significant difference ($P < 0.05$)

At baseline, IL-1β was significantly less in H compared to diseased sites ($P < 0.0001$) and was not significantly different between SRP+C and SRP sites ($P = 0.13$). At 3-months post-therapy, IL-1β remained significantly less in H compared to diseased sites ($P \leq 0.0001$) and was not significantly different between SRP+C and SRP sites ($P = 0.72$).

At baseline, IL-1ra was significantly less in H compared to SRP+C ($P = 0.007$) and SRP sites ($P = 0.02$). There was no significant difference between SRP+C and SRP sites ($P = 0.68$). At 3-months post-therapy, IL-1ra remained significantly lower in H compared to SRP+C sites ($P = 0.0001$); however, IL-1ra was not significantly different in H compared to SRP sites ($P = 0.08$). IL-1ra was significantly greater in SRP+C than SRP sites ($P = 0.007$) and the mean IL-ra levels increased only in SRP+C sites between examinations.

At baseline, the anti-inflammatory index was significantly higher in H compared to SRP+C ($P = 0.001$) and SRP sites ($P = 0.0006$). There was no significant difference between SRP+C and SRP sites ($P = 0.81$). At 3-months post-therapy, the anti-inflammatory index remained significantly higher in H compared to SRP+C ($P = 0.0006$) and SRP sites ($P = 0.0002$). There was no significant difference between SRP+C and SRP sites ($P = 0.74$).

***Outcomes based on BOP resolution:***

Clinical measures and cytokine levels comparing treated, diseased sites with BOP resolution to non-resolution of all sites combined (i.e., test and control) are shown in Tables 7-10.

**Table 7:** Clinical outcomes by BOP resolution

| Clinical Parameter | BOP Post-therapy | N | Baseline (SEM) | Mean Change (SEM) |
|---|---|---|---|---|
| **PD (mm)** | (+) | 51 | 5.84 (0.15) | -0.45 (0.14) |
| | (-) | 33 | 5.92 (0.18) | -0.88 (0.17) |
| **CAL (mm)** | (+) | 51 | 4.85 (0.40) | -0.20 (0.16) |
| | (-) | 33 | 4.97 (0.46) | -0.58 (0.20) |
| **PI** | (+) | 51 | 1.70 (0.11) | 0.02 (0.11) |
| | (-) | 33 | 1.40 (0.14) | 0.07 (0.14) |

No statistically significant difference between BOP (+) and BOP (-) for each clinical parameter

**Table 8:** IL-1β levels by BOP resolution

| BOP Post-therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **(+)** | 31 | 148 (18) pg | 113 (17) pg |
| **(-)** | 19 | 113 (21) pg | 79 (19) pg |

No statistically significant difference between BOP (+) and BOP (-)

**Table 9:** IL-1ra levels by BOP resolution

| BOP Post-therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **(+)** | 31 | 18,986 (3,323) pg | 17,875 (3,170) pg |
| **(-)** | 19 | 20,561 (3,601) pg | 19,135 (3,569) pg |

No statistically significant difference between BOP (+) and BOP (-)

**Table 10:** Anti-inflammatory index (IL-1ra/IL-1β ratio) by BOP resolution

| BOP Post-therapy | N | BASELINE Mean (SEM)/30-sec | 3 MO POST-THERAPY Mean (SEM)/30-sec |
|---|---|---|---|
| **(+)** | 31 | 243 (173)[a] | 191 (49)[a] |
| **(-)** | 19 | 462 (180)[a] | 330 (54)[b] |

Dissimilar superscripts within each column indicate significant difference ($P < 0.05$)

The mean baseline PD, CAL, and PI were not significantly different in sites where BOP resolved or did not resolve. The differences in changes between resolved and unresolved sites in PD reduction ($P = 0.0552$), CAL gain ($P = 0.14$), and PI change ($P = 0.79$) were not significant; although, there was a definite trend in greater PD reduction in sites where BOP resolved.

The mean baseline levels of IL-1β, IL-1ra, and anti-inflammatory index were not significantly different when comparing sites where BOP resolved or remained unresolved. The differences in mean post-therapy levels of IL-1β and IL-1ra remained insignificant; however, the post-therapy anti-inflammatory index was significantly greater in sites where BOP resolved ($P = 0.02$).

**DISCUSSION**

This single-blinded, randomized controlled trial compared clinical and cytokine measurements of two therapies, SRP+C (test) and SRP (control), for bleeding pockets ≥ 5 mm in periodontal maintenance patients over a 3-month period. Every precaution was taken to eliminate any bias by compartmentalizing the various aspects of this study protocol as follows: blinded examiner (CR) collected all data, clinician (MB) who was blinded to chemical pocket disinfection assignment performed all scaling and root planing, and clinician (WK) randomized/performed chemical pocket disinfection therapy. All of the participants in this study were maintenance patients on a 3- to 4-month recall program and had previously received periodontal therapy. The baseline clinical and cytokine measurements for SRP+C and SRP sites were similar.

The current study demonstrated that inflamed pockets treated with SRP+C or SRP showed PD reduction, CAL gain, and BOP reduction 3-months post-therapy in a periodontal maintenance population. No significant differences were shown when comparing clinical measurements of SRP+C to SRP. Only one other known study about sodium hypochlorite has also compared clinical measures of SRP+C to SRP (Forgas & Gound 1987). It investigated untreated patients with generalized moderate periodontitis rather than maintenance patients. The sample size was smaller (n=10) than the current study's sample. Nevertheless, the results showed clinical improvement in all treated sites compared to untreated control sites, but no differences between treatments in PD, CAL, or gingival index at 2-months post-therapy. The Loe and Silness gingival index was used to evaluate inflammation, which is broader in its criteria (evaluates gingival appearance in addition to BOP) compared to only recording BOP as present or absent. BOP is important to assess as its presence indicates histologic inflammation (Greenstein et al. 1981, Amato et al. 1986) and presence of subgingival bacteria (Mombelli et al.

2000, Wilson et al. 2008). Ideally, the goal for each maintenance patient would be to eliminate BOP at all sites to reduce the risk of progressive breakdown (Chaves et al. 1990, Lang et al. 1990).

BOP reduction was not significantly different between SRP+C and SRP groups (-48.8% vs. -30.2%, respectively), but SRP+C did show a trend ($P$ = 0.08) of reducing inflammation more effectively. This may be attributed to more effective bacterial reduction by the disinfective properties of sodium hypochlorite (Adcock et al. 1983) or improved healing of the attachment apparatus with elimination of inflamed pocket tissues (Kalkwarf et al. 1982). Studies have shown that sites with BOP correlated with higher percentages of periodontal pathogens (Armitage et al. 1982, Demmer et al. 2008) which sodium hypochlorite has been shown to effectively reduce in periodontal pockets (Adcock et al. 1983). However, a previous study has shown that SRP+C similarly reduces subgingival bacterial load compared to SRP (Forgas & Gound 1987). Healing after curettage is also similar to scaling and root planing with the formation of a long junctional epithelium (Waerhaug 1978, Caton & Zander 1979, Caton et al. 1980).

The CAL data had conflicting results as the CAL change between SRP+C and SRP was not significantly different ($P$ = 0.43), but when evaluating each therapy independently, the CAL change for SRP+C was significantly improved ($P$ = 0.01) while SRP was not significantly improved ($P$ = 0.18). This may be explained by the greater BOP resolution trend in SRP+C and less probe penetration into the non-inflamed attachment (Listgarten et al. 1976, Magnusson & Listgarten 1980). If so, PD also should have reflected greater reduction in SRP+C, but was essentially identical to results in SRP.

Plaque control was poor in the recruited maintenance patients. Plaque was present at most treated sites at both baseline and post-therapy exams, resulting in no improvement in PI. This may help explain why BOP did not resolve in over half of the sites for both treatments.

Most of the studied sites were found in posterior, interproximal sites which have been shown to be difficult for patients to clean effectively (Cumming & Loe 1973, Prasad et al. 2011). Improved PI may have resulted in better clinical results for both groups.

Analysis of the baseline cytokine data showed significantly higher amounts of IL-1β and IL-1ra for SRP+C and SRP sites compared to H sites, as expected. This finding has been reported by other studies (Ishihara et al. 1997, Goutoudi et al. 2004, Holmlund et al. 2004, Gilowski et al. 2014).

Post-therapy, IL-1β levels in SRP+C and SRP sites showed a decreasing trend. Several studies have demonstrated a reduction in IL-1β that also had a reduction in clinical inflammation after therapy (Hou et al. 1995, Tsai et al. 1995, Engebretson et al. 2002, Goutoudi et al. 2004). The reduction in BOP is a clinical reflection of the decrease of IL-1β. This can also be interpreted as a decreased risk of further periodontal destruction based on the study by Reinhardt et al. (2010), who showed a higher risk of future breakdown when IL-1β increased. In the current study, IL-1ra increased in SRP+C sites and decreased in SRP sites, which resulted in a significant difference between therapies ($P = 0.007$). Holmlund et al. (2004) showed a decrease in IL-1ra after surgical therapy in moderate to advanced periodontitis. Toker et al. (2008) showed in aggressive periodontitis that IL-1ra did not significantly change six weeks after therapy.

The overall decrease in IL-1β but increase in IL-1ra in sites treated with SRP+C is important and may relate to the enhanced BOP reduction compared to SRP. The imbalance between IL-1β and IL-1ra has been evaluated in many other inflammatory diseases including arthritis, inflammatory bowel disease, graft-versus-host disease, osteoporosis, and diabetes (Arend 2002). The relationship of these opposing cytokines can be expressed by the anti-inflammatory index (IL-1ra/IL-1β ratio). Despite the decrease in IL-1β and increase in IL-1ra for SRP+C sites, the anti-inflammatory index did not increase. This is because the individual index

values for each patient determine the average index, rather than using the average IL-1β and IL-1ra values for all patients to determine the average index.

To better understand how IL-1β and IL-1ra relate to clinical inflammation and BOP, the cytokines were evaluated relative to BOP resolution rather than by therapy. Both IL-1β and IL-1ra were reduced in similar amounts post-therapy, regardless of BOP resolution. Evaluating the anti-inflammatory index, a significantly higher value in the sites where BOP resolved was observed compared to sites with persistent BOP post-therapy ($P = 0.02$). This index was still significantly less than the H sites, but may help explain why BOP was not present 3-months post-therapy.

The cytokine measures were highly variable for each patient. With the large range in measured values, it is difficult to understand how to effectively utilize IL-1β and IL-1ra in everyday practice. If cytokine analyses were to be used, a baseline for each patient would need to be established and sites of interest can be monitored with the hope that trends may be detected. Clinicians may be able to predict risk of further breakdown or predictability of favorable pocket resolution. Further research is needed to explore how the detection of cytokines in GCF can be implemented in practice to enhance the treatment of periodontitis; however, it may be found to be unsuitable for use in clinical practice.

The use of SRP+C in practice to treat inflamed pockets in maintenance patients is safe and should be considered. The adjunctive application of sodium hypochlorite utilizing a strict protocol ensures safe outcomes and does not cause any harm to the patient, as shown in the current study and in the Forgas & Gound study (1987) with similar clinical outcomes. Histologically, application of sodium hypochlorite in a pocket/sulcus results in normal healing (Johnson & Waerhaug 1956, Kalkwarf et al. 1982). The potential benefit of enhanced inflammation reduction may outweigh the minimal risks that it poses.

SRP+C may be comparable to other adjunctive therapies in its benefits and limitations. Currently, the most-studied adjunctive therapies commonly being used are minocycline (Williams et al. 2001), doxycycline (Bogren et al. 2008), chlorhexidine chips (Jeffcoat et al. 1998), povidone-iodine (Hoang et al. 2003), and lasers (Schwarz et al. 2008). The purpose of these therapies is to reduce subgingival bacterial flora and clinical signs of periodontitis. The American Academy of Periodontology addressed the limitations of adjunctive therapies and stated that local adjuncts resulted in modest improvements (PD reduction of 0.25-0.5 mm) in the clinical outcomes of pockets ≥ 5 mm. The AAP also stated that the use of adjuncts is not proven to "reduce the need for surgery or improve long-term tooth retention", or to be cost effective (AAP Statement 2006). Many adjunctive therapies utilize locally-delivered antibiotics. The medical community is calling for more judicious antibiotic use because of the potential for promoting resistant bacteria and creating a "superbug" (Laxminarayan et al. 2013). Povidone-iodine and lasers potentially avoid the pitfalls of antibiotics; however, the benefits of povidone-iodine (Hoang et al. 2003, Kruck et al. 2012) and lasers (Schwarz et al. 2008, Sgolastra et al. 2013, Slot et al. 2014) are either limited or unproven. Sodium hypochlorite also does not have the risk of developing bacterial resistance (McDonnell & Russell 1999) and would provide another tool in the armamentarium of clinicians. Due to its limitations, adjuncts should be considered by clinicians only for localized recurrent and/or residual inflamed pockets ≥ 5 mm after conventional scaling and root planing (AAP Statement 2006). Fortunately, the incidence of recurrent periodontitis is very infrequent and is usually controllable with scaling and root planing alone (Kaldahl et al. 1996b). Further studies should be undertaken to compare SRP+C to other adjunctive forms of therapy.

When analyzing the data based upon whether or not BOP resolved and ignoring the type of therapy given, some interesting findings were discovered. No matter the resolution of

BOP, both groups had improvements in PD reduction and CAL gain. Interestingly, plaque was present in a majority of the study sites, including sites where BOP resolved. It was expected that plaque would play a more influential role in the healing of inflamed pockets. Although not statistically significant, there was a trend for PD reduction to be greater when BOP resolved compared to BOP not resolving ($P = 0.0552$). CAL gain did not have as strong a trend ($P = 0.14$), but was still greater in sites where BOP resolved. The greater PD reduction and CAL gain can be expected in sites where BOP resolved due to less probe penetration into less inflamed attachment tissues (Listgarten et al. 1976, Magnusson & Listgarten 1980). These greater clinical improvements may also be a clinical manifestation of the significantly greater anti-inflammatory index.

This study has several limitations. Patients were observed for only a period of three months and a longer duration would be ideal. Also, only two inflammatory cytokines were observed. IL-1β was selected due to the large number of published periodontitis studies on its pro-inflammatory effects and proven role in inflammation. IL-1ra was utilized due to its direct antagonizing effects on IL-1β. Literature on IL-1ra in periodontitis-related studies is limited. Numerous other cytokines are involved in the inflammatory process and IL-1β and IL-1ra only represent a fraction of all that is occurring in an inflamed pocket. The etiology of periodontal disease is initiated by bacteria and was not evaluated in this study. Also, the history of each study site was not investigated. Although all sites were inflamed, some may have been experiencing active attachment loss while others may have been periodontally stable, which may influence the response and inflammatory condition of the pockets.

**CONCLUSIONS**

SRP+C does not enhance the clinical benefits of scaling and root planing in the treatment of periodontal maintenance patients with inflamed pockets. However, the adjunctive application of concentrated sodium hypochlorite may have a positive effect on the inflammatory condition of the pocket. Future studies of SRP+C with larger samples and longer observation periods are needed to further assess the anti-microbial and anti-inflammatory effects. Additionally, further studies comparing SRP+C to other adjunctive therapies, such as locally-delivered antibiotics and lasers, should be pursued.

**BIBLIOGRAPHY**

Adcock JE, Berry WC Jr, Kalkwarf KL. Effect of sodium hypochlorite solution on the subgingival microflora of juvenile periodontitis lesions. Pediatric Dent 1983;5(3):190-194.

Amato R, Caton J, Polson A, Espeland M. Interproximal gingival inflammation related to the conversion of a bleeding to a nonbleeding state. J Periodontol 1986;57(2):63-68.

American Academy of Periodontology. The pathogenesis of periodontal diseases (position paper). J Periodontol 1999;70(4):457-470.

American Academy of Periodontology. Parameter on chronic periodontitis with slight to moderate loss of periodontal support. J Periodontol 2000;71(Suppl. 5):853-855.

American Academy of Periodontology. The American Academy of Periodontology statement regarding gingival curettage. J Periodontol 2002;73(10):1229-1230.

American Academy of Periodontology. Diagnosis of periodontal diseases (position paper). J Periodontol 2003;74(8):1237-1247.

American Academy of Periodontology. American Academy of Periodontology statement on local delivery of sustained or controlled release antimicrobials as adjunctive therapy in the treatment of periodontitis. J Periodontol 2006;77(8):1458-1458.

American Dental Association. Accepted Dental Therapeutics. Chicago American Dental Association, 1984;326.

Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. J Clin Invest 1990;85(5):1694-1697.

Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev 2002;13(4-5):323-340.

Armitage GC, Dickinson WR, Jenderseck RS, Levine SM, Chambers DW. Relationship between the percentage of subgingival spirochetes and the severity of periodontal disease. J Periodontol 1982;53(9):550-556.

Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4(1):1-6.

Axelsson P, Lindhe J. The significance of maintenance care in the treatment of periodontal disease. J Clin Periodontol 1981;8(4):281-294.

Becker W, Becker BE, Berg LE. Periodontal treatment without maintenance. A retrospective study in 44 patients. J Periodontol 1984a;55(9):505-509.

Becker W, Berg L, Becker BE. The long term evaluation of periodontal treatment and maintenance in 95 patients. Int J Perio Rest Dent 1984b;4(2):54-71.

Becker W, Becker BE, Caffesse R, Kerry G, Ochsenbein C, Morrison E, Prichard J. A longitudinal study comparing scaling, osseous surgery, and modified Widman procedures: results after 5 years. J Periodontol 2001;72(12):1675-1684.

Bogren A, Teles RP, Torresyap G, Haffajee AD, Socransky SS, Wennstrom JL. Locally delivered doxycycline during supportive periodontal therapy: a 3-year study. J Periodontol 2008;79(5):827-835.

Bonito AJ, Lux L, Lohr KN. Impact of local adjuncts to scaling and root planing in periodontal disease therapy: a systematic review. J Periodontol 2005;76(8):1227-1236.

Bostrom L, Linder LE, Bergstrom J. Smoking and GCF levels of IL-1 beta and IL-1ra in periodontal disease. J Clin Periodontol 2000;27(4):250-255.

Box KF. Periodontal disease and treatment. J Ont Dental Assoc, 1952;29:194-199.

Box KF. Further report on antiformin. J Ont Dental Assoc, 1953;30:84-92.

Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, Nuki G, Pavelka K, Rau R, Rozman B, Watt I, Williams B, Aitchison R, McCabe D, Musikic P. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998;41(12):2196-2204.

Bresnihan B, Newmark R, Robbins S, Genant HK. Effects of anakinra monotherapy on joint damage in patients with rheumatoid arthritis. Extension of a 24-week randomized, placebo-controlled trial. J Rheumatol 2004;31(6):1103-1111.

Buduneli N, Buduneli E, Cetin EO, Kirilmaz L, Kutukculer N. Clinical findings and gingival crevicular fluid prostaglandin E2 and interleukin-1-beta levels following initial periodontal treatment and short-term meloxicam administration. Expert Opin Pharmacother 2010;11(11):1805-1812.

Caliskan MK, Turkun M, Alper S. Allergy to sodium hypochlorite during root canal therapy: a case report. Int Endod J 1994;27(3):163-167.

Carter MJ, Jones S, Camp NJ, Cox A, Mee J, Warren B, Duff GW, Lobo AJ, di Giovine FS. Functional correlates of the interleukin-1 receptor antagonist gene polymorphism in the colonic mucosa in ulcerative colitis. Genes Immun 2004;5(1):8-15.

Casini-Raggi V, Kam L, Chong YJ, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. J Immunol 1995;154(5):2434-40.

Caton JG, Zander HA. The attachment between tooth and gingival tissues after periodic root planing and soft tissue curettage. J Periodontol 1979;50(9):462-466.

Caton J, Nyman S, Zander H. Histometric evaluation of periodontal surgery. II. Connective tissue attachment levels after four regenerative procedures. J Clin Periodontol 1980;7(3):224-231.

Chan LS, Hammerberg C, Kang K, Sabb P, Tavakkol A, Cooper KD. Human dermal fibroblast interleukin-1 receptor antagonist (IL-1ra) and interleukin-1 beta (IL-1 beta) mRNA and protein are co-stimulated by phorbol ester: implication for a homeostatic mechanism. J Invest Dermatol 1992;99(3):315-322.

Chaves ES, Caffesse RG, Morrison EC, Stults DL. Diagnostic discrimination of bleeding on probing during maintenance periodontal therapy. Am J Dent 1990;3(4):167-170.

Chiplunkar S, Langhorne J, Kaufmann SHE. Stimulation of B cell growth and differentiation by murine recombinant interleukin-1. J Immunol 1986;137(12):3748-3752.

Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. J Clin Periodontol 2002;29 Suppl 2:6-16.

Cobb CM. Lasers in Periodontics: a review of the literature. J Periodontol 2006;77(4):545-564.

Cochran DL. Inflammation and bone loss in periodontal disease. J Periodontol 2008;79(8 Suppl):1569-1576.

Crane AB. A practicable root canal technique. Philadelphia: Lea & Febinger, 1920.

Cumming BR, Loe H. Consistency of plaque distribution in individuals without special home care instruction. J Periodontol Res 1973;8(2):94-100.

Demmer RT, Papapanou PN, Jacobs DR Jr, Desvarieux M. Bleeding on probing differentially relates to bacterial profiles: the oral infections and vascular disease epidemiology study. J Clin Periodontol 2008;35(6):479-486.

Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 beta. J Immunol 1985;135(4):2562-2568.

Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991;77(8):1627-1652.

Dinarello CA. Overview of the interleukin-1 family of ligands and receptors. Semin Immunol 2013;25(6):389-393.

Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, Taylor GW, Page RC, Beck JD, Genco RJ. Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. J Periodontol 2015;86(5):611-622.

Engebretson SP, Grbic JT, Singer R, Lamster IB. GCF IL-1β profiles in periodontal disease. J Clin Periodontol 2002;29(1):48-53.

Estrela C, Estrela CRA, Barbin EL, Spano JCE, Marchesan MA, Pecora JD. Mechanism of action of sodium hypochlorite. Braz Dent J 2002;13(2):113-117.

Forgas LB, Gound S. The effects of antiformin-citric acid chemical curettage on the microbial flora of the periodontal pocket. J Periodontol 1987;58(3):153-158.

Garcia Canas P, Khouly I, Sanz J, Loomer PM. Effectiveness of systemic antimicrobial therapy in combination with scaling and root planing in the treatment of periodontitis: a systematic review. J Am Dent Assoc 2015;146(3):150-163.

Garrison SW, Nichols FG. LPS-elicited secretory responses in monocytes: Altered release of PGE2 but not IL-1 beta in patients with adult periodontitis. J Periodontal Res 1989;24(2):88-95.

Gilowski L, Wiench R, Plocica I, Krzeminski TF. Amount of interleukin-1β and interleukin-1 receptor antagonist in periodontitis and healthy patients. Arch Oral Biol 2014;59(7):729-734.

Gilthorpe MS, Zamzuri AT, Griffiths GS, Maddick IH, Eaton KA, Johnson NW. Unification of the “burst” and “linear” theories of periodontal disease progression: A multilevel manifestation of the same phenomenon. J Dent Res 2003;82(3):200-205.

Glickman I, Patur B. Histologic study of the effect of antiformin on the soft tissue wall of periodontal pockets in human beings. J Am Dent Assoc 1955;51(4):420-424.

Goldman HM. Curettage by ultrasonic instrument. Preliminary report. Oral Surg Oral Med Oral Pathol 1960;13:43-53.

Goodson JM, Tanner AC, Haffajee AD, Somberger GC, Socransky SS. Patterns of progression and regression of advanced destructive periodontal disease. J Clin Periodontol 1982;9(6):472-481.

Goutoudi P, Diza E, Arvanitidou M. Effect of periodontal therapy on crevicular fluid interleukin-1β and interleukin-10 levels in chronic periodontitis. J Dent 2004;32(7):511-520.

Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. J Periodontol 2003;74(3):391-401.

Graves D. Cytokines that promote periodontal tissue destruction. J Periodontol 2008;79(8 Suppl):1585-1591.

Greenstein G, Caton J, Polson AM. Histologic characteristics associated with bleeding after probing and visual signs of inflammation. J Periodontol 1981;52(8):420-425.

Griffen AL, Lyons SR, Becker MR, Moeschberger ML, Leys EJ. Porphyromonas gingivalis strain variability and periodontitis. J Clin Microbiol 1999;37(12):4028-4033.

Gruaz-Chatellard D, Baumberger C, Saurat JH, Dayer JM. Interleukin 1 receptor antagonist in human epidermis and cultured keratinocytes. FEBS Lett 1991;294(1-2):137-140.

Haffajee AD, Uzel NG, Arguello EI, Torresyap G, Guerrero DM, Socransky SS. Clinical and microbiological changes associated with the use of combined antimicrobial therapies to treat "refractory" periodontitis. J Clin Periodontol 2004;31(10):869-877.

Hagaman DD, Okayama Y, D'Ambrosio C, Prussin C, Gilfillan AM, Metcalfe DD. Secretion of interleukin-1 receptor antagonist from human mast cells after immunoglobulin E-mediated activation and after segmental antigen challenge. Am J Respir Cell Mol Biol 2001;25(6):685-691.

Hecker F. Pyorrhea alveolaris. St. Louis, C.V. Mosby Co., 1913, p.145.

Hirschfeld L. Subgingival curettage in periodontal treatment. J Am Dent Assoc 1952;44(3):301-314.

Hoang T, Jorgensen MG, Keim RG, Pattison AM, Slots J. Povidone-iodine as a periodontal pocket disinfectant. J Peridontal Res 2003;38(3):311-317.

Holmlund A, Hanstrom L, Lerner UH. Bone resorbing activity and cytokine levels in gingival crevicular fluid before and after treatment of periodontal disease. J Clin Periodontol 2004;31(6):475-482.

Horowitz MC. Cytokines and estrogen in bone: anti-osteoporotic effects. Science 1993;260(5108):626-627.

Hou LT, Liu CM, Rossomando EF. Crevicular interleukin-1β in moderate and severe periodontitis patients and the effect of phase I periodontal treatment. J Clin Periodontol 1995;22(2):162-167.

Hung HC, Douglass CW. Meta-analysis of the effect of scaling and root planing, surgical treatment and antibiotic therapies on periodontal probing depth and attachment loss. J Clin Periodontol 2002;29(11):975-986.

Hunter HA. A study of tissues treated with antiformin-citric acid. J Canad Dent Assoc 1955;21-344.

Ishihara Y, Nishihara T, Kuroyanagi T, Shirozu N, Yamagishi E, Ohguchi M, Koide M, Ueda N, Amano K, Noguchi T. Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. J Periodont Res 1997;32(6):524-529.

Jeffcoat MK, Bray KS, Ciancio SG, Dentino AR, Fine DH, Gordon JM, Gunsolley JC, Killoy WJ, Lowenguth RA, Magnusson NI, Offenbacher S, Palcanis KG, Proskin HM, Finkelman RD, Flashner M. Adjunctive use of a subgingival controlled-release chlorhexidine chip reduces probing depth and improves attachment level compared with scaling and root planing alone. J Periodontol 1998;69(9):989-997.

Johnson RE, Waerhaug J. Effect of antiformin on gingival tissues. J Periodontol 1956;27:24-28.

Juge-Aubry CE, Somm E, Chicheportiche R, Burger D, Pernin A, Cuenod-Pittet B, Quinodoz P, Giusti V, Dayer JM, Meier CA. Regulatory effects of interleukin (IL)-1, interferon-beta, and IL-4 on the production of IL-1 receptor antagonist by human adipose tissue. J Clin Endocrinol Metab 2004;89(6):2652-2658.

Kabashima H, Nagata K, Hashiguchi I, Toriya Y, Iijima T, Maki K, Maeda K. Interleukin-1 receptor antagonist and interleukin-4 in gingival crevicular fluid of patients with inflammatory periodontal disease. J Oral Pathol Med 1996;25(8):449-455.

Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK. Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. J Periodontol 1996a;67(2):93-102.

Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK. Long-term evaluation of periodontal therapy: II. Incidence of sites breaking down. J Periodontol 1996b;67(2):103-108.

Kalkwarf KL, Tussing GJ, Davis MJ. Histologic evaluation of gingival curettage facilitated by sodium hypochlorite solution. J Periodontol 1982;53(2):63-70.

Karimbux NY, Saraiya VM, Elangovan S, Allareddy V, Kinnunen T, Kornman KS, Duff GW. Interleukin-1 gene polymorphisms and chronic periodontitis in adult whites: a systematic review and meta-analysis. J Periodontol 2012;83(11):1407-1419.

Kim DM, Koszeghy KL, Badovinac RL, Kawai T, Hosokawa I, Howell TH, Karimbux NY. The effect of aspirin on gingival crevicular fluid levels of inflammatory and anti-inflammatory mediators in patients with gingivitis. J Periodontol 2007;78(8):1620-1626.

Kristensen M, Deleuran B, Eedy DJ, Feldmann M, Breathnach SM, Brennan FM. Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1 alpha in normal and psoriatic skin. Decreased expression of IRAP in psoriatic lesional epidermis. Br J Dermatol 1992;127(4):305-311.

Kruck C, Eick S, Knofler GU, Purschwitz RE, Jentsch HF. Clinical and microbiologic results 12 months after scaling and root planing with different irrigation solutions in patients with moderate chronic periodontitis: a pilot randomized trial. J Periodontol 2012;83(3):312-320.

Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. J Clin Periodontol 1990;17(10):714-721.

Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H, Greko C, So AD, Bigdeli M, Tomson G, Woodhouse W, Ombaka E, Peralta AQ, Qamar FN, Mir F, Kariuki S, Bhutta ZA, Coates A, Bergstrom R, Wright GD, Brown ED, Cars O. Antibiotic resistance-the need for global solutions. Lancet Infect Dis 2013;13(12):1057-1098.

Lindhe J, Ranney R, Lamster I, Charles A, Chung CP, Flemmig T, Kinane D, Listgarten M, Loe H, Schoor R, Seymour G, Somerman M. Consensus Report: Chronic periodontitis. Ann Periodontol 1999;4(1):38.

Listgarten M, Mao R, Robinson PJ. Periodontal probing and the relationship of the probe tip to periodontal tissues. J Periodontol 1976;47(9):511-513.

Listgarten MA, Lindhe J, Hellden L. Effect of tetracycline and/or scaling on human periodontal disease. Clinical, microbiological, and histological observations. J Clin Periodontol 1978;5(4):246-271.

Lobene RR, Soparkar PM, Hein JW, Quigley GA. A study of the effects of antiseptic agents and a pulsating irrigating device on plaque and gingivitis. J Periodontol. 1972:43(9);564-568.

Magnusson I, Listgarten MA. Histologic evaluation of probing depth following periodontal treatment. J Clin Periodontol 1980;7(1):26-31.

Magnusson I, Lindhe J, Yoneyama T, Liljenberg B. Recolonization of a subgingival microbiota following scaling in deep pockets. J Clin Periodontol 1984;11(3):193-207.

Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1α and -1β in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. J Periodont Res 1990;25(3):156-163.

Matsuki Y, Yamamoto T, Hara K. Detection of inflammatory cytokine messenger RNA (mRNA)-expressing cells in human inflamed gingival by combined in situ hybridization and immunohistochemistry. Immunology 1992;76(1):42-47.

McColl SR, Paquin R, Menard C, Beaulieu AD. Human neutrophils produce high levels of the interleukin 1 receptor antagonist in response to granulocyte/macrophage colony-stimulating factor and tumor necrosis factor alpha. J Exp Med 1992;176(2):593-598.

McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999;12(1):147-179.

Mergenhagen SE. Thymocyte activating factor(s) in human gingival fluids. J Dent Res 1984;63(3):461-464.

Miller S, Sorrin S. The action and use of sodium sulphide solution as an epithelial solvent. Dent Cosmos 1927;69:1113-1116.

Mizel SB. Interleukin 1 and T-cell activation. Immunol Today 1987;8(11):330-332.

Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K. Interleukin 1 beta, interleukin 6, beta 2-microglobulin, and transforming growth factor-alpha in gingival crevicular fluid from human periodontal disease. Arch Oral Biol 1999;44(6):535-539.

Mombelli A, Schmid B, Rutar A, Lang NP. Persistence patterns of Porphyromonas gingivalis, Prevotella intermedia/nigrescens, and Actinobacillus actinomycetemcomitans after mechanical therapy of periodontal disease. J Periodontol 2000;71(1):14-21.

Moore WE, Moore LV. The bacteria of periodontal diseases. Periodontol 2000 1994;5:66-77.

Moskow BS. The response of the gingival sulcus to instrumentation: a histological investigation. J Periodontol 1964;35:112-126.

Nadler H. Removal of crevicular epithelium by ultrasonic curettes. J Periodontol 1962;33:220.

Nishimine D, O'Leary TJ. Hand instrumentation versus ultrasonics in the removal of endotoxins from root surfaces. J Periodontol 1979;50(7):345-349.

Nyman S, Rosling B, Lindhe J. Effect of professional tooth cleaning on healing after periodontal surgery. J Clin Periodontol 1975;2(2):80-86.

Nyman S, Lindhe J, Rosling B. Periodontal surgery in plaque-infected dentitions. J Clin Periodontol 1977;4(4):240-249.

O'Hoy PY, Messer HH, Palamara JE. The effect of cleaning procedures on fracture properties and corrosion of NiTi files. Int Endod J 2003;36(11):724-732.

Oosterwaal PJ, Matee MI, Mikx FH, van't Hof MA, Renggli HH. The effect of subgingival debridement with hand and ultrasonic instruments on the subgingival microflora. J Clin Periodontol 1987;14(9):528-533.

Opp MR, Postlethwaite AE, Seyer JM, Krueger JM. Interleukin 1 receptor antagonist blocks somnogenic and pyrogenic responses to an interleukin 1 fragment. Proc Natl Acad Sci USA 1992;89(9):3726-3730.

Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin 1. Immunology Today 1986;7(2):45-56.

Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. Oral Microbiol Immunol 2006;21(4):256-260.

Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. Lab Invest 1976;34(3):235-249.

Page RC, Schroeder HE. Current status of the host response in chronic marginal periodontitis. J Periodonol 1981;52(9):477-491.

Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 1997;14:216-248.

Perrier S, Kherratia B, Deschaumes C, Ughetto S, Kemeny JL, Baudet-Pommel M, Sauvezie B. IL-1ra and IL-1 production in human oral mucosal epithelial cells in culture: differential modulation by TGF-beta1 and IL-4. Clin Exp Immunol 2002;127(1):53-59.

Perrier S, Darakhshan F, Hajduch E. IL-1 receptor antagonist in metabolic diseases: Dr Jekyll or Mr Hyde? FEBS Lett 2006;580(27):6289-6294.

Prasad KV, Sreenivasan PK, Patil S, Chhabra KG, Javali SB, DeVizio W. Removal of dental plaque from different regions of the mouth after 1-minute episode of mechanical oral hygiene. Am J Dent 2011;24(1):60-64.

Preiss DS, Meyle J. Interleukin-1β concentration of gingival crevicular fluid. J Periodontol 1994;65(5):423-428.

Ramfjord SP. Maintenance care for treated periodontitis patients. J Clin Periodontol 1987;14(8):433-437.

Ramfjord SP, Caffesse RG, Morrison EC, Hill RW, Kerry GJ, Appleberry EA, Nissle RR, Stults DL. 4 modalities of periodontal treatment compared over 5 years. J Clin Periodontol 1987;14(8):445-452.

Rawlinson A, Dalati MH, Rahman S, Walsh TF, Fairclough AL. Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. J Clin Periodontol 2000;27(10):738-743.

Reinhardt RA, Stoner JA, Golub LM, Lee H, Nummikoski PV, Sorsa T, Payne JB. Association of gingival crevicular fluid biomarkers during periodontal maintenance with subsequent progressive periodontitis. J Periodontol 2010;81(2):251-259.

Richette P, Francois M, Vicaut E, Fitting C, Bardin T, Corvol M, Savouret JF, Rannou F. A high interleukin 1 receptor antagonist/IL-1beta ratio occurs naturally in knee osteoarthritis. J Rheumatol 2008;35(8):1650-1654.

Roux-Lombard P, Modoux C, Dayer JM. Production of interleukin-1 (IL-1) and a specific IL-1 inhibitor during human monocyte-macrophage differentiation: influence of GM-CSF. Cytokine 1989;1(1):45-51.

Sauder DN, Mounessa NC, Katz SJ, Dinarello CA, Gallin JJ. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. J Immunol 1984;132(2):828-832.

Schwarz F, Aoki A, Becker J, Sculean A. Laser application in non-surgical periodontal therapy: a systematic review. J Clin Periodontol 2008;35(8 Suppl):29-44.

Seckinger P, Williamson K, Balavoine JF, Mach B, Mazzei G, Shaw A, Dayer JM. A urine inhibitor of interleukin 1 activity affects both interleukin 1 alpha and 1 beta but not tumor necrosis factor alpha. J Immunol 1987;139(5):1541-1545.

Sgolastra F, Gatto R, Petrucci A, Monaco A. Effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: a systematic review and meta-analysis. J Periodontol 2012;83(10):1257-1269.

Sgolastra F, Severino M, Gatto R, Monaco A. Effectiveness of diode laser as adjunctive therapy to scaling root planing in the treatment of chronic periodontitis: a meta-analysis. Lasers Med Sci 2013;28(5):1393-1402.

Shaw C. Chemical aspects of antiformin chemosurgery. J Ont Dent Assoc 1953;30:47.

Shiloah J, Hovious LA. The role of subgingival irrigations in the treatment of periodontitis. J Periodontol 1993;64(9):835-843.

Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odont Scand 1964;22:112-135.

Slot DE, Jorritsma KH, Cobb CM, Van der Weijden FA. The effect of the thermal diode laser (wavelength 808-980 nm) in non-surgical periodontal therapy: a systematic review and meta-analysis. J Clin Periodontol 2014;41(7):681-692.

Slots J, Mashimo P, Levine MJ, Genco RJ. Periodontal therapy in humans. I. Microbiological and clinical effects of a single course of periodontal scaling and root planing, and of adjunctive tetracycline therapy. J Periodontol 1979;50(10):495-509.

Slots J. Selection of antimicrobial agents in periodontal therapy. J Periodontol Res 2002;37(5):389-398.

Socransky SS, Haffajee AD, Teles R, Wennstrom JL, Lindhe J, Bogren A, Hasturk H, Van Dyke T, Wang X, Goodson JM. Effect of periodontal therapy on the subgingival microbiota over a 2-year monitoring period. I. Overall effect and kinetics of change. J Clin Periodontol 2013;40(8):771-780.

Stone S, Ramfjord SP, Waldron J. Scaling and gingival curettage: a radioautographic study. J Periodontol 1966;37:415.

Toker H, Poyraz O, Eren K. Effect of periodontal treatment on IL-1beta, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis. J Clin Periodontol 2008;35(6):507-513.

Trombelli L, Scapoli C, Carrieri A, Giovannini G, Calura G, Farina R. Interleukin-1 beta levels in gingival crevicular fluid and serum under naturally occurring and experimentally induced gingivitis. J Clin Periodontol 2010;37(8):697-704.

Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. J Periodontol 1995;66(10):852-859.

Van Dyke TE, Schweinebraten M, Cianciola LJ, Offenbacher S, Genco RJ. Neutrophil chemotaxis in families with localized juvenile periodontitis. J Periodontal Res 1985;20(5):503-514.

Van Dyke TE. The management of inflammation in periodontal disease. J Periodontol 2008;79(8 Suppl):1601-1608.

Vieira EM, O'Leary TJ, Kafrawy AH. The effect of sodium hypochlorite and citric acid solution on healing of periodontal pockets. J Periodontol 1982;53(2):71-80.

Waerhaug J. Microscopic demonstration of tissue reaction incident to removal of subgingival calculus. J Periodontol 1955;26:26.

Waerhaug J, Loe H. Effect of phenol camphor on gingival tissues. J Periodontol 1958;29:59-66.

Waerhaug J. Healing of the dentoepithelial junction following subgingival plaque control: I. As observed in human biopsy material. J Periodontol 1978;1:1-8.

Williams RC, Paquette DW, Offenbacher S, Adams DF, Armitage GC, Bray K, Caton J, Cochran DL, Drisko CH, Fiorellini JP, Giannobile WV, Grossi S, Guerrero DM, Johnson GK, Lamster IB, Magnusson I, Oringer RJ, Persson GR, Van Dyke TE, Wolff LF, Santucci EA, Rodda BE, Lessem J. Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. J Periodontol 2001;72(11):1535-1544.

Wilson TG Jr, Glover ME, Malik AK, Schoen JA, Dorsett D. Tooth loss in maintenance patients in a private periodontal practice. J Periodontol 1987;58(4):231-235.

Wilson TG, Harrel SK, Nunn ME, Francis B, Webb K. The relationship between the presence of tooth-borne subgingival deposits and inflammation found with a dental endoscope. J Periodontol 2008;79(11):2029-2035.

Yukna RA. A clinical and histologic study of healing following the excisional new attachment procedure in rhesus monkeys. J Periodontol 1976;47(12):701-709.

**Appendix A: Plaque index**

0 = No plaque.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface.

2 = Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.

3 = Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

(As described in Silness & Loe 1964)

**Appendix B: Concentrated sodium hypochlorite formula**

100 mL Purex bleach (6% active chlorine)
7.8 g sodium hydroxide
19.0 g sodium carbonate (anhydrous)

pH of approximately 14 is produced.

(As described in Kalkwarf et al. 1982)

**Appendix C: Raw clinical data for SRP+C**

| Pt # | Test site | PD Ex 1 | PD Ex 2 | PD Δ | REC Ex 1 | CAL Ex 1 | REC Ex 2 | CAL Ex 2 | CAL Δ | BOP Ex 1 | BOP Ex 2 | BOP Δ | PI Ex 1 | PI Ex 2 | PI Δ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 2ML | 5 | 6 | 1 | -3 | 2 | -2 | 4 | 2 | 1 | 1 | 0 | 1 | 2 | 1 |
| 2 | 31ML | 5 | 4 | -1 | -2 | 3 | -2 | 2 | -1 | 1 | 0 | -1 | 1 | 2 | 1 |
| 3 | 13DF | 5 | 4 | -1 | -3 | 2 | -3 | 1 | -1 | 1 | 1 | 0 | 0 | 1 | 1 |
| 3 | 31ML | 6 | 6 | 0 | -2 | 4 | -2 | 4 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 4 | 14DB | 5 | 4 | -1 | -1 | 4 | -2 | 2 | -2 | 1 | 0 | -1 | 1 | 2 | 1 |
| 5 | 14DB | 7 | 6 | -1 | 1 | 8 | 2 | 8 | 0 | 1 | 0 | -1 | 2 | 2 | 0 |
| 5 | 18DL | 5 | 3 | -2 | 0 | 5 | 1 | 4 | -1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 6 | 31ML | 6 | 6 | 0 | -2 | 4 | -2 | 4 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 7 | 24DB | 6 | 4 | -2 | 2 | 8 | 3 | 7 | -1 | 1 | 1 | 0 | 0 | 2 | 2 |
| 7 | 32ML | 5 | 4 | -1 | -2 | 3 | -3 | 1 | -2 | 1 | 1 | 0 | 2 | 2 | 0 |
| 8 | 20DL | 5 | 5 | 0 | -2 | 3 | -2 | 3 | 0 | 1 | 1 | 0 | 3 | 2 | -1 |
| 8 | 18ML | 6 | 6 | 0 | -3 | 3 | -3 | 3 | 0 | 1 | 1 | 0 | 3 | 2 | -1 |
| 9 | 12ML | 7 | 6 | -1 | -2 | 5 | -2 | 4 | -1 | 1 | 0 | -1 | 1 | 2 | 1 |
| 9 | 14ML | 5 | 5 | 0 | -2 | 3 | -2 | 3 | 0 | 1 | 1 | 0 | 2 | 3 | 1 |
| 9 | 31ML | 5 | 5 | 0 | -2 | 3 | -2 | 3 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 10 | 2ML | 5 | 6 | 1 | 3 | 8 | 4 | 10 | 2 | 1 | 1 | 0 | 2 | 2 | 0 |
| 11 | 3MB | 9 | 9 | 0 | -3 | 6 | -2 | 7 | 1 | 1 | 1 | 0 | 1 | 1 | 0 |
| 13 | 15DL | 5 | 4 | -1 | -3 | 2 | -2 | 2 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 13 | 19ML | 7 | 6 | -1 | -4 | 3 | -5 | 1 | -2 | 1 | 1 | 0 | 2 | 1 | -1 |
| 15 | 4ML | 8 | 7 | -1 | -3 | 5 | -3 | 4 | -1 | 1 | 0 | -1 | 1 | 1 | 0 |
| 15 | 2DL | 6 | 3 | -3 | -1 | 5 | 1 | 4 | -1 | 1 | 1 | 0 | 1 | 1 | 0 |
| 16 | 3DL | 7 | 6 | -1 | -3 | 4 | -2 | 4 | 0 | 1 | 0 | -1 | 1 | 1 | 0 |
| 17 | 14MB | 5 | 5 | 0 | 2 | 7 | 1 | 6 | -1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 17 | 19B | 6 | 7 | 1 | 1 | 7 | 1 | 8 | 1 | 1 | 1 | 0 | 2 | 1 | -1 |
| 18 | 19DL | 5 | 5 | 0 | -2 | 3 | -2 | 3 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 20 | 29DL | 5 | 5 | 0 | -4 | 1 | -3 | 2 | 1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 23 | 4L | 6 | 5 | -1 | 3 | 9 | 3 | 8 | -1 | 1 | 0 | -1 | 0 | 0 | 0 |
| 23 | 14ML | 6 | 4 | -2 | -2 | 4 | -2 | 2 | -2 | 1 | 1 | 0 | 2 | 2 | 0 |
| 23 | 30ML | 6 | 6 | 0 | -2 | 4 | -2 | 4 | 0 | 1 | 0 | -1 | 2 | 1 | -1 |
| 24 | 5ML | 7 | 7 | 0 | 1 | 8 | 1 | 8 | 0 | 1 | 0 | -1 | 0 | 0 | 0 |
| 24 | 18L | 6 | 6 | 0 | 5 | 11 | 4 | 10 | -1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 26 | 31ML | 6 | 5 | -1 | 0 | 6 | 0 | 5 | -1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 28 | 2ML | 6 | 7 | 1 | 0 | 6 | 0 | 7 | 1 | 1 | 0 | -1 | 2 | 0 | -2 |
| 28 | 11DL | 7 | 5 | -2 | 2 | 9 | 3 | 8 | -1 | 1 | 0 | -1 | 1 | 2 | 1 |
| 28 | 30DL | 6 | 4 | -2 | 1 | 7 | 1 | 5 | -2 | 1 | 0 | -1 | 2 | 2 | 0 |
| 30 | 15DL | 6 | 6 | 0 | 0 | 6 | 0 | 6 | 0 | 1 | 1 | 0 | 3 | 2 | -1 |
| 30 | 30MB | 7 | 6 | -1 | 0 | 7 | 0 | 6 | -1 | 1 | 0 | -1 | 2 | 3 | 1 |
| 31 | 5ML | 6 | 3 | -3 | 0 | 6 | 1 | 4 | -2 | 1 | 0 | -1 | 2 | 2 | 0 |
| 31 | 14MB | 6 | 6 | 0 | 2 | 8 | 2 | 8 | 0 | 1 | 1 | 0 | 2 | 0 | -2 |
| 32 | 3B | 5 | 4 | -1 | 4 | 9 | 4 | 8 | -1 | 1 | 0 | -1 | 0 | 0 | 0 |
| 32 | 12DL | 5 | 5 | 0 | -1 | 4 | -1 | 4 | 0 | 1 | 0 | -1 | 2 | 2 | 0 |

**Appendix D: Raw clinical data for SRP**

| Pt # | Test site | PD Ex 1 | PD Ex 2 | PD Δ | REC Ex 1 | CAL Ex 1 | REC Ex 2 | CAL Ex 2 | CAL Δ | BOP Ex 1 | BOP Ex 2 | BOP Δ | PI Ex 1 | PI Ex 2 | PI Δ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 31DL | 5 | 5 | 0 | -2 | 3 | -2 | 3 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 3 | 2ML | 5 | 4 | -1 | -2 | 3 | -2 | 2 | -1 | 1 | 0 | -1 | 1 | 0 | -1 |
| 3 | 14DL | 5 | 5 | 0 | -3 | 2 | -4 | 1 | -1 | 1 | 1 | 0 | 1 | 2 | 1 |
| 4 | 19MB | 5 | 3 | -2 | -2 | 3 | -1 | 2 | -1 | 1 | 0 | -1 | 0 | 1 | 1 |
| 5 | 3DB | 6 | 5 | -1 | 2 | 8 | 2 | 7 | -1 | 1 | 1 | 0 | 0 | 1 | 1 |
| 5 | 30DL | 5 | 5 | 0 | -2 | 3 | -3 | 2 | -1 | 1 | 0 | -1 | 1 | 1 | 0 |
| 6 | 9ML | 6 | 6 | 0 | -3 | 3 | -3 | 3 | 0 | 1 | 0 | -1 | 2 | 1 | -1 |
| 6 | 19DB | 6 | 6 | 0 | -1 | 5 | 2 | 8 | 3 | 1 | 0 | -1 | 2 | 3 | 1 |
| 7 | 11ML | 5 | 6 | 1 | 0 | 5 | 1 | 7 | 2 | 1 | 1 | 0 | 2 | 2 | 0 |
| 7 | 18ML | 6 | 5 | -1 | -2 | 4 | -1 | 4 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 8 | 2ML | 5 | 4 | -1 | -3 | 2 | -2 | 2 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 8 | 13MB | 6 | 7 | 1 | -2 | 4 | -2 | 5 | 1 | 1 | 1 | 0 | 2 | 1 | -1 |
| 9 | 3DL | 8 | 8 | 0 | -2 | 6 | -2 | 6 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 9 | 19DL | 6 | 5 | -1 | -3 | 3 | -3 | 2 | -1 | 1 | 1 | 0 | 1 | 2 | 1 |
| 9 | 26DB | 6 | 4 | -2 | 2 | 8 | 2 | 6 | -2 | 1 | 1 | 0 | 2 | 2 | 0 |
| 10 | 18MB | 5 | 5 | 0 | 2 | 7 | 2 | 7 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 12 | 2ML | 5 | 4 | -1 | -2 | 3 | -2 | 2 | -1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 13 | 3DL | 5 | 5 | 0 | -2 | 3 | -1 | 4 | 1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 14 | 12MB | 8 | 8 | 0 | -1 | 7 | -1 | 7 | 0 | 1 | 1 | 0 | 1 | 2 | 1 |
| 14 | 15DL | 6 | 5 | -1 | -2 | 4 | -1 | 4 | 0 | 1 | 1 | 0 | 1 | 2 | 1 |
| 14 | 17ML | 5 | 4 | -1 | -3 | 2 | -3 | 1 | -1 | 1 | 1 | 0 | 2 | 1 | -1 |
| 16 | 15ML | 5 | 5 | 0 | -2 | 3 | -3 | 2 | -1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 16 | 30DL | 5 | 4 | -1 | -2 | 3 | 1 | 5 | 2 | 1 | 1 | 0 | 1 | 1 | 0 |
| 17 | 30DL | 5 | 4 | -1 | 2 | 7 | 2 | 6 | -1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 19 | 13DL | 7 | 7 | 0 | -4 | 3 | -4 | 3 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 21 | 3ML | 5 | 4 | -1 | 2 | 7 | 2 | 6 | -1 | 1 | 0 | -1 | 1 | 2 | 1 |
| 21 | 31DL | 7 | 3 | -4 | -1 | 6 | 0 | 3 | -3 | 1 | 0 | -1 | 1 | 1 | 0 |
| 23 | 2ML | 5 | 4 | -1 | 2 | 7 | 2 | 6 | -1 | 1 | 1 | 0 | 1 | 2 | 1 |
| 23 | 18MB | 5 | 4 | -1 | 0 | 5 | 1 | 5 | 0 | 1 | 1 | 0 | 3 | 3 | 0 |
| 23 | 23DB | 9 | 9 | 0 | 1 | 10 | 2 | 11 | 1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 24 | 14DB | 7 | 8 | 1 | 2 | 9 | 2 | 10 | 1 | 1 | 1 | 0 | 2 | 0 | -2 |
| 25 | 5ML | 7 | 4 | -3 | -2 | 5 | -3 | 1 | -4 | 1 | 1 | 0 | 1 | 1 | 0 |
| 26 | 4ML | 6 | 6 | 0 | -2 | 4 | -2 | 4 | 0 | 1 | 0 | -1 | 0 | 0 | 0 |
| 26 | 14ML | 5 | 5 | 0 | -4 | 1 | -4 | 1 | 0 | 1 | 0 | -1 | 2 | 2 | 0 |
| 27 | 3DL | 5 | 4 | -1 | -1 | 4 | 0 | 4 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 29 | 2DL | 7 | 5 | -2 | -2 | 5 | -1 | 4 | -1 | 1 | 1 | 0 | 1 | 2 | 1 |
| 29 | 15DL | 5 | 5 | 0 | 0 | 5 | 0 | 5 | 0 | 1 | 1 | 0 | 2 | 3 | 1 |
| 30 | 13DL | 8 | 7 | -1 | 2 | 10 | 2 | 9 | -1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 31 | 2ML | 6 | 6 | 0 | 3 | 9 | 3 | 9 | 0 | 1 | 1 | 0 | 2 | 1 | -1 |
| 31 | 15DL | 7 | 8 | 1 | -1 | 6 | -1 | 7 | 1 | 1 | 1 | 0 | 2 | 2 | 0 |
| 32 | 5ML | 5 | 4 | -1 | -1 | 4 | 0 | 4 | 0 | 1 | 0 | -1 | 2 | 1 | -1 |
| 32 | 18ML | 5 | 4 | -1 | -1 | 4 | -1 | 3 | -1 | 1 | 0 | -1 | 2 | 2 | 0 |
| 32 | 31ML | 6 | 6 | 0 | -2 | 4 | -1 | 5 | 1 | 1 | 1 | 0 | 2 | 2 | 0 |

**Appendix E: Raw IL-1β and IL-1ra cytokine data in diseased sites treated with SRP+C**

| Pt # | Test site | IL-1β Ex 1 (pg/30-sec) | IL-1β Ex 2 (pg/30-sec) | IL-1β change | IL-1ra Ex 1 (pg/30-sec) | IL-1ra Ex 2 (pg/30-sec) | IL-1ra change | IL-1ra/IL-1β Ex 1 | IL-1ra/IL-1β Ex 2 |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 13DF | 130.922 | 16.858 | -114.064 | 16772.100 | 1901.187 | -14870.913 | 128 | 113 |
| 3 | 31ML | 159.136 | 10.106 | -149.030 | 15706.160 | 1966.582 | -13739.578 | 99 | 195 |
| 4 | 14DB | 87.997 | 22.046 | -65.951 | 6838.541 | 1476.117 | -5362.424 | 78 | 67 |
| 5 | 14DB | 89.916 | 79.753 | -10.163 | 2744.788 | 10350.280 | 7605.492 | 31 | 130 |
| 5 | 18DL | 69.306 | 25.883 | -43.423 | 7335.546 | 1234.154 | -6101.392 | 106 | 48 |
| 7 | 24DB | 185.147 | 262.000 | 76.853 | 861.400 | 41041.090 | 40179.690 | 5 | 157 |
| 7 | 32ML | 93.327 | 183.454 | 90.127 | 1188.377 | 28193.740 | 27005.363 | 13 | 154 |
| 8 | 20DL | 60.760 | 23.539 | -37.221 | 18588.170 | 7078.401 | -11509.769 | 306 | 301 |
| 8 | 18ML | 44.364 | 25.171 | -19.193 | 18657.560 | 18707.860 | 50.300 | 421 | 743 |
| 9 | 12ML | 262.000 | 255.563 | -6.437 | 19300.050 | 19155.150 | -144.900 | 74 | 75 |
| 9 | 14ML | 262.000 | 182.055 | -79.945 | 29253.320 | 25611.020 | -3642.300 | 112 | 141 |
| 9 | 31ML | 262.000 | 262.000 | 0 | 13823.250 | 49252.800 | 35429.550 | 53 | 188 |
| 10 | 2ML | 147.555 | 48.715 | -98.840 | 25081.240 | 21703.840 | -3377.400 | 170 | 446 |
| 13 | 15DL | 189.437 | 144.757 | -44.680 | 40974.870 | 52166.630 | 11191.760 | 216 | 360 |
| 16 | 3DL | 262.000 | 103.048 | -158.952 | 64550.420 | 17890.720 | -46659.700 | 246 | 174 |
| 17 | 14MB | 118.196 | 119.853 | 1.657 | 20535.920 | 13361.300 | -7174.620 | 174 | 111 |
| 23 | 4L | 70.780 | 42.772 | -28.008 | 16168.760 | 17614.770 | 1446.010 | 228 | 412 |
| 23 | 14ML | 246.243 | 123.166 | -123.077 | 22532.340 | 12997.920 | -9534.420 | 92 | 106 |
| 23 | 30ML | 145.573 | 26.677 | -118.896 | 20968.620 | 14515.640 | -6452.980 | 144 | 544 |
| 24 | 5ML | 4.586 | 50.819 | 46.233 | 19323.540 | 52977.700 | 33654.160 | 4214 | 1042 |
| 26 | 31ML | 67.703 | 70.780 | 3.077 | 14245.000 | 19475.320 | 5230.320 | 210 | 275 |
| 30 | 30MB | 81.352 | 216.376 | 135.024 | 2448.139 | 13882.270 | 11434.131 | 30 | 64 |
| 31 | 5ML | 262.000 | 75.564 | -186.436 | 16688.350 | 52158.140 | 35469.790 | 64 | 690 |
| 31 | 14MB | 262.000 | 230.032 | -31.968 | 18993.890 | 43527.160 | 24533.270 | 72 | 189 |
| 32 | 12DL | 194.568 | 31.112 | -163.456 | 32235.940 | 13190.610 | -19045.330 | 166 | 424 |

**Appendix F: Raw IL-1β and IL-1ra cytokine data in diseased sites treated with SRP**

| Pt # | Test site | IL-1β Ex 1 (pg/30-sec) | IL-1β Ex 2 (pg/30-sec) | IL-1β change | IL-1ra Ex 1 (pg/30-sec) | IL-1ra Ex 2 (pg/30-sec) | IL-1ra change | IL-1ra/IL-1β Ex 1 | IL-1ra/IL-1β Ex 2 |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 2ML | 15.578 | 4.634 | -10.944 | 1691.922 | 1103.363 | -588.559 | 109 | 238 |
| 3 | 14DL | 6.055 | 23.893 | 17.838 | 2025.438 | 4451.608 | 2426.170 | 335 | 186 |
| 4 | 19MB | 167.664 | 79.469 | -88.195 | 29592.160 | 9330.106 | -20262.054 | 176 | 117 |
| 5 | 3DB | 30.858 | 62.341 | 31.483 | 5327.907 | 894.098 | -4433.809 | 173 | 14 |
| 5 | 30DL | 66.321 | 24.959 | -41.362 | 5471.777 | 2045.057 | -3426.720 | 83 | 82 |
| 7 | 11ML | 9.182 | 84.372 | 75.190 | 1449.959 | 1613.447 | 163.488 | 158 | 19 |
| 7 | 18ML | 98.870 | 22.685 | -76.185 | 6655.434 | 1103.363 | -5552.071 | 67 | 49 |
| 8 | 2ML | 178.714 | 68.530 | -110.184 | 28591.080 | 14088.140 | -14502.940 | 160 | 206 |
| 8 | 13MB | 168.846 | 44.830 | -124.016 | 27200.390 | 10965.390 | -16235.000 | 161 | 245 |
| 9 | 3DL | 43.276 | 197.363 | 154.087 | 9371.534 | 12035.220 | 2663.686 | 217 | 61 |
| 9 | 19DL | 187.261 | 159.832 | -27.429 | 13132.530 | 15910.740 | 2778.210 | 70 | 100 |
| 9 | 26DB | 219.897 | 105.750 | -114.147 | 11041.860 | 32564.490 | 21522.630 | 50 | 308 |
| 10 | 18MB | 179.413 | 146.855 | -32.558 | 32763.160 | 25875.920 | -6887.240 | 183 | 176 |
| 13 | 3DL | 262.000 | 147.011 | -114.989 | 48656.790 | 27597.730 | -21059.060 | 186 | 188 |
| 16 | 15ML | 125.798 | 121.667 | -4.131 | 22763.420 | 16792.640 | -5970.780 | 181 | 138 |
| 17 | 30DL | 158.512 | 138.078 | -20.434 | 7621.595 | 15173.670 | 7552.075 | 48 | 110 |
| 23 | 2ML | 155.908 | 118.196 | -37.712 | 15544.880 | 14356.440 | -1188.440 | 100 | 121 |
| 23 | 18MB | 262.000 | 262.000 | 0 | 33949.350 | 24029.650 | -9919.700 | 130 | 92 |
| 23 | 23DB | 64.784 | 147.545 | 82.761 | 19475.320 | 27897.710 | 8422.390 | 301 | 189 |
| 24 | 14DB | 8.847 | 45.928 | 37.081 | 13236.520 | 15014.470 | 1777.950 | 1496 | 327 |
| 26 | 4ML | 53.975 | 29.202 | -24.773 | 48485.770 | 16871.830 | -31613.940 | 898 | 578 |
| 30 | 13DL | 262.000 | 262.000 | 0 | 19965.650 | 18028.690 | -1936.960 | 76 | 69 |
| 31 | 2ML | 193.314 | 118.483 | -74.831 | 6723.280 | 18877.590 | 12154.310 | 35 | 159 |
| 31 | 15DL | 85.040 | 28.255 | -56.785 | 13023.140 | 16235.100 | 3211.960 | 153 | 575 |
| 32 | 5ML | 34.874 | 42.957 | 8.083 | 13509.830 | 17515.980 | 4006.150 | 387 | 408 |

**Appendix G: Raw IL-1β and IL-1ra cytokine data in healthy sites**

| Pt # | Test site | IL-1β Ex 1 (pg/30-sec) | IL-1β Ex 2 (pg/30-sec) | IL-1β change | IL-1ra Ex 1 (pg/30-sec) | IL-1ra Ex 2 (pg/30-sec) | IL-1ra change | IL-1ra/IL-1β Ex 1 | IL-1ra/IL-1β Ex 2 |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 4DF | 3.212 | 7.263 | 4.051 | 3326.807 | 2221.625 | -1105.182 | 1036 | 306 |
| 4 | 30MB | 34.838 | 47.630 | 12.792 | 5079.404 | 5576.409 | 497.005 | 146 | 117 |
| 5 | 4DB | 9.466 | 2.218 | -7.248 | 1005.27 | 966.033 | -39.237 | 106 | 436 |
| 7 | 6MB | 7.903 | 12.878 | 4.975 | 939.874 | 11769.360 | 10829.486 | 119 | 914 |
| 8 | 28ML | 21.908 | 14.681 | -7.227 | 15224.060 | 8010.774 | -7213.286 | 695 | 546 |
| 9 | 20ML | 86.091 | 63.557 | -22.534 | 15876.180 | 12540.340 | -3335.840 | 184 | 197 |
| 10 | 30MB | 31.154 | 39.546 | 8.392 | 16118.640 | 12597.040 | -3521.600 | 517 | 319 |
| 13 | 29ML | 2.093 | 10.485 | 8.392 | 8199.768 | 12893.130 | 4693.362 | 3918 | 1230 |
| 16 | 13ML | 1.352 | 0.878 | -0.474 | 8083.885 | 4454.082 | -3629.803 | 5979 | 5073 |
| 17 | 4MB | 26.519 | 16.894 | -9.625 | 13613.500 | 12122.310 | -1491.190 | 513 | 718 |
| 23 | 21ML | 4.981 | 45.139 | 40.158 | 13910.670 | 11612.860 | -2297.810 | 2793 | 257 |
| 24 | 3MB | 12.239 | 14.527 | 2.288 | 12987.300 | 17291.740 | 4304.440 | 1061 | 1190 |
| 26 | 21ML | 14.054 | 9.557 | -4.497 | 23405.770 | 11480.190 | -11925.580 | 1665 | 1201 |
| 30 | 5MB | 0.878 | 1.291 | 0.413 | 4952.915 | 9915.561 | 4962.646 | 5641 | 7681 |
| 31 | 12MB | 64.695 | 5.124 | -59.571 | 21062.960 | 8301.686 | -12761.274 | 326 | 1620 |
| 32 | 28MB | 6.865 | 1.919 | -4.946 | 10222.970 | 3513.264 | -6709.706 | 1489 | 1831 |

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 AT THE NEBRASKA MEDICAL CENTER 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.**

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

## ADULT CONSENT - CLINICAL BIOMEDICAL

**Title of this Research Study**
Using Chemical Pocket Disinfection as an Adjunct to Non-surgical Maintenance Therapy of Inflamed Periodontal Pockets

**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 a periodontal maintenance patient, at least 30 years of age, and have one or more sites with at least 5 mm pocket depths (spaces between gum and teeth) that are inflamed around your teeth. If you are pregnant or plan to become pregnant during this study, you may not be included in this study.

**What is the reason for doing this research study?**
This study tries to determine whether the additional use of a disinfectant solution with "cleaning" (known as scaling and root planing) will result in the reduction of inflammation and pocket depths in patients who are being maintained on a regular basis but have pocket depths that are at least 5 mm with bleeding. When a pocket is bleeding, it is inflamed. It is usually "cleaned" with periodontal instruments to re-establish health. Disinfectant solution therapy is currently being used in the UNMC College of Dentistry but has never been evaluated in a research setting. This research is trying to see if additional disinfectant solution therapy is beneficial.

**What will be done during this research study?**
If you decide to participate in this study, teeth with at least 5mm pockets with bleeding on probing will be chosen for the study. You will have a clinical exam of the areas of pocketing as is routinely done in dental offices. Fluid from the pockets surrounding the tooth (gingival crevicular fluid) will also be collected by absorptive paper for analysis of inflammation. Then, the teeth will be "cleaned." The sites will then be randomized by flipping a coin to determine which receive the additional disinfectant solution treatment. These therapies ("cleaning" and disinfectant solution therapy) are routinely used in the UNMC College of Dentistry and private practice.



Subject's Initials______________

IRB Approved
Valid until 11/21/2014

Three months later, you will return for the same clinical examination and collection of fluid from the periodontal pockets being tested. We will try to do this in conjunction with your next 3-month periodontal maintenance appointment. The additional time for each examination (initial and 3-month post-therapy data collection) will be approximately 30 minutes. The therapy ("cleaning" or "cleaning" plus disinfectant solution) will take approximately 5-10 minutes per site.

**What are the possible risks of being in this research study?**
The risks from the treatment modalities and collection of gingival fluid are extremely small and will be similar to when you have your teeth cleaned and treated with one of the above modalities in any dental office. Slight temporary tenderness or soreness of the gums could be expected when you have routine cleaning and/or disinfectant solution therapy. There are no known specific risks with these procedures. There are also no known risks for gingival crevicular fluid sampling. It is also possible that you could have a side effect that has not occurred before.

**What are the possible benefits to you?**
Periodontal health may improve for those sites treated with "scaling and root planing" and "scaling and root planing + chemical pocket disinfection" with a reduction in pocket depth, reduction in inflammation, and/or gain in clinical tooth attachment. You may also not get any benefit from being in this research study.

**What are the possible benefits to other people?**
The information from this study may guide dentists and dental hygienists as to what modality of treatment is more effective and therefore more desirable for future patient care in these bleeding and inflamed periodontal pockets.

**What are the alternatives to being in this research study?**
Instead of being in this research study, you can choose not to participate and will only receive the standard method of cleaning as originally planned.

**What will being in this research study cost you?**
There is no added cost to you to be in this study. There will be no charge for the therapy of the study sites of interest (scaling and root planing and chemical pocket disinfection therapies) or for any of the study examinations.

However, you are expected to pay for your regular periodontal maintenance just as when you have your routine periodontal maintenance treatment done. If any additional visits are required for the study, no charges will be incurred.



Subject's Initials______________

IRB Approved
Valid until 11/21/2014

**Will you be paid for being in this research study?**
You will not be paid to be in this research study. However, when you return for the subsequent 3-month follow-up clinical examination and periodontal maintenance, you will receive a 30% discount on your regular periodontal maintenance therapy (not including other treatment such as x-rays).

**Who is paying for this research?**
The research is being paid for by the Periodontal Development Fund, College of Dentistry, University of Nebraska Medical Center.

**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.



Subject's Initials______________

IRB Approved
Valid until 11/21/2014

Your PHI will be used only for the purpose(s) described in the section.

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 HIPAA Privacy Rule requires the following groups to protect your PHI:
    - Your health insurance company

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?**

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: 40th & Holdrege Sts., Lincoln, NE 68583

**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 medical care or your relationship with the investigator or the Institution. Your doctor 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.



**Subject's Initials**_______________

IRB Approved
Valid until 11/21/2014

Deciding to withdraw will otherwise not affect your care or your relationship with the investigator or this institution. You will not lose any benefits to which you are entitled.

You may be taken off the study if you do not follow instructions of the investigator or the research team.

You may also be taken off the study if

- you do not come in for your 3 month follow-up appointment

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 complaints 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



IRB Approved
Valid until 11/21/2014

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**
Riggs, Chad Makoto
phone: 402-472-1316
degree: DDS

**Secondary**
Byarlay, Matthew (Matt) Ryan
phone: 402-472-1316
degree: DDS, MS

**Faculty Advisor**
Kaldahl, Wayne Berwyn
phone: 402-472-1273
degree: DDS

**Participating Personnel**
Christiansen, Mary
Reinhardt, Richard (Rick) August



IRB Approved
Valid until 11/21/2014

phone: 402-472-6770
degree: BS, RDH

phone: 402-472-1287
alt #: 402-472-1287
degree: DDS, MS, PhD



Subject's Initials______________

IRB Approved
Valid until 11/21/2014