Spring 2003

Sulphur By-Product: A Potential Indicator of Early Dental Plaque-Induced Gingival Disease Activity

Aleksandra Pavolotskaya
Old Dominion University

SULPHUR BY-PRODUCT: A POTENTIAL INDICATOR OF EARLY DENTAL PLAQUE-INDUCED GINGIVAL DISEASE ACTIVITY

by

Aleksandra Pavolotskaya, RDH
BEd. May 1991, Odessa Pedagogical Institute

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE IN
DENTAL HYGIENE

OLD DOMINION UNIVERSITY
May 2003

Approved by:

Gayle McCombs (Director)

Michele Darby (Member)

Kenneth Marinak (Member)

ABSTRACT

SULPHUR BY-PRODUCT: A POTENTIAL INDICATOR OF EARLY DENTAL PLAQUE-INDUCED GINGIVAL DISEASE ACTIVITY

Aleksandra Pavolotskaya
Old Dominion University, 2003
Director: Gayle McCombs

The purpose of this study was to determine the relationship between volatile sulfide compounds and gingival health status, and to recognize if volatile sulfide compounds can detect early dental plaque-induced gingival disease, using a 21-day experimental gingivitis model. A split-mouth design with randomly selected quadrants of the mandibular arch enabled 39 subjects, 19-62 years of age, to serve as their own controls. A baseline full-mouth periodontal probing assessment was obtained to verify gingival health and enrollment status. At baseline and at three subsequent appointments, gingival inflammation, bleeding on probing, and sulfide levels were measured using the Gingival Index and the Diamond Probe/Perio 2000 System®. For three weeks, subjects refrained from brushing and flossing one randomly selected quadrant of the mandibular arch. The Pearson correlation test was used to determine the relationship between sulfide levels and gingival health status. The Wilcoxon matched-pairs signed rank test was used to compare the differences in gingival inflammation, bleeding on probing and sulfide levels between the hygiene and non-hygiene side at baseline, day 7, 14, and 21. Gingival Index, bleeding on probing and sulfide level scores showed statistically significant differences from baseline until the end of the study between both the hygiene and non-hygiene side, however, the strength of the correlation was stronger for the non-hygiene side. The Diamond Probe/Perio 2000 System® was able to detect sites with elevated sulfide levels, which suggest that volatile sulfide compounds may

serve as a useful marker in detecting early dental plaque-induced gingivitis. Whether sulfur by-product is a contributor to the disease process, or merely a correlate remains unanswered.

ACKNOWLEDGEMENTS

Many people have contributed to the successful completion of this research. I extend sincere thanks to my thesis committee for revising and editing this manuscript, for their patience and mentorship. Special thanks to my family for their unconditional love and support.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Periodontal disease includes a group of inflammatory conditions of the periodontal tissues broadly categorized as gingivitis and periodontitis. Inflammation of the gingiva induced by plaque is the most common form of gingivitis (Page, 1985). Experimental research in humans confirmed bacterial plaque as an etiology of gingival disease (Loe, Jensen & Theilade, 1965). Moreover, according to the American Academy of Periodontology (1999), the initiation and progression of most forms of gingivitis are dependent upon the presence and persistence of bacterial plaque. As Gram-negative bacteria cross the epithelial barrier into the underlying connective tissue of the periodontium, an inflammatory reaction is initiated leading to connective tissue breakdown.

Gram-negative bacteria have the potential of generating volatile sulfur compounds (VSC), e.g., hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3), as by-products of their metabolism (Persson, Edlund, Claesson & Carlsson, 1990). It is also believed that these by-products of bacterial metabolism are factors in the etiology of periodontal disease (Yaegaki & Suetaka, 1989). Studies demonstrate that even in low concentrations, VSC are highly toxic to periodontal tissue and may contribute to the etiology of gingivitis (Ng & Tonzetich, 1984; Johnson & Tonzetich, 1985). Tonzetich (1978) proposed that VSC might alter the permeability of affected cells, and facilitate the transmission of toxic metabolites into underlying connective tissue and contribute to the progression of periodontal disease. Yaegaki and Suetaka (1989) found that concentrations of VSC precursors increased with the severity

of periodontal disease. The ratio of CH_3SH and H_2S significantly increased in patients with periodontal disease in proportion to the bleeding index and probing depth. In addition, the amounts of H_2S and CH_3SH in infected periodontal pockets were considered high. However, the validity of VSC as an indicator of plaque-induced gingivitis and its relationship to oral health is still unknown.

In an attempt to develop objective measures of disease activity, studies have tested saliva, blood, plaque, and gingival crevicular fluid (GCF) (Tonzetich, 1978; Yaegaki & Sanada, 1992; Kaldahl, Kalkwarf, Patil & Molver, 1990; Lang, Joss, Orsanic, Gusberti & Siegrist, 1986). Bleeding, a classic sign of inflammation, and periodontal probing have long been used as standard measures of disease activity (Lang et al, 1986).

Bleeding on probing (BOP) and the Gingival Index (GI) have been used to clinically characterize the degree of clinical gingival inflammation. According to Zhou (2001), sulfide levels (SUL) correlate with BOP and GI in persons who have not brushed over a 14 day period. In addition, SUL increase with the rise of BOP, suggesting that SUL may contribute to the progression of gingival inflammation. Since it appears that bacterial by-products contribute to the initiation and progression of gingival disease, SUL might be a valid indicator of disease activity. As plaque bacteria and bacterial by-products interact with the host, inflammation and tissue destruction results, leading to the clinical signs and symptoms of gingivitis (Van Dyke, Offenbacher, Pihlstrom, Putt & Trummel, 1998).

Periodontitis is not an inevitable consequence of gingivitis; however, gingivitis always precedes periodontitis (Van Dyke et al, 1998). It is becoming increasingly apparent that the traditional clinical criteria are inadequate for determining active disease

sites, monitoring quantitatively the response to therapy or measuring the degree of susceptibility to future breakdown. Refinements in sampling of blood, saliva, plaque and gingival crevicular fluid (GCF) and the availability of more analytical techniques for detection of disease-associated bacteria in subgingival plaque give significant cause for optimism (Fine & Mandel, 1986). The presence of SUL in the gingival sulcus should attract the attention of the clinician, since research suggests a correlation between SUL and plaque-induced gingivitis.

Statement of the Problem

The purpose of this study was to answer the following questions:

1. Are sulfide levels within the gingival sulcus capable of detecting early dental plaque-induced gingival disease in a 21-day experimental gingivitis model?
   - If gingival health is the baseline, is there any relationship between sulfide levels and gingival inflammation?
   - If gingival health is the baseline, is there any relationship between sulfide levels and bleeding on probing?
2. Which periodontal parameter appears to be the most reliable clinical indicator of early dental plaque-induced gingival disease: sulfide levels, bleeding on probing or gingival inflammation?

Significance of the Problem

The cytotoxicity of H_2S and CH_3SH strongly suggests that VSC may play an etiologic role in the gingival disease process (Fine & Mandel, 1986). Both H_2S and CH_3SH are capable of inducing a change in the structure of the crevicular epithelium, initiating the destructive inflammatory process (Ng & Tonzetich, 1984). Rizzo (1967)

indicated that detectable hydrogen sulfide is produced in the deepest portion of periodontal pockets in humans. Studies reveal that sulfur by-products are associated with periodontal disease (Johnson, Yaegaki & Tonzetich, 1992; Morita & Wang, 2001). Research by Morita and Wang (2001) suggests a positive correlation between sulcular sulfide levels and severity of periodontal disease. This finding supports the early work of Fine and Mandel (1986) who suggested that hydrogen sulfide generated by causative bacteria in periodontal disease may indicate the initiation or progression of periodontal disease.

The Diamond Probe/Perio 2000 System®[1] is a dental device that intended to detect and monitor periodontal bacterial activity. The system is an in-vitro device for measuring probing pocket depths, bleeding on probing, and sulfides within gingival crevicular fluid as indicators of disease activity. Traditional periodontal probing is a retrospective analysis of disease, whereas the Diamond Probe/Perio 2000 System® measures current disease activity and provides the opportunity for earlier periodontal disease assessment. The system reduces uncertainty about the presence of Gram-negative bacteria in the gingival sulci by detecting different forms of sulfides (S^-, HS^-, H_2S, and CH_3SH). The presence of relative sulfide concentrations in individual sulcus sites alerts the practitioner and the patient to relative levels of bacterial activity. In addition, the system provides visible and audible indicators when sulfides are detected. If determined effective, the Diamond Probe/Perio 2000 System® may improve clinical decision-making in periodontal assessment.

[1] Registered Trademark of Diamond General Development Corporation, Ann Arbor, MI

Definition of Terms

The following key variables are defined:

Diamond Probe/Perio 2000 System® Research Model - A data collection device based on advanced ion-selective electrode technology that measures sulfide concentration, probing depth, and bleeding on probing. The research model consists of an electronic control unit that provides immediate audible, visual, and quantitative indicators of relative sulfide levels.

Diamond Probe/Perio 2000 System® Office Model - A data collection device based on advanced ion-selective electrode technology that measures sulfide concentration, probing depth, and bleeding on probing. The office model provides immediate, adjunctive information on bacterial activity by indicating the presence or absence of sulfides, and with visual and audible responses to alert practitioners and motivate patients. The chairside system is designed for routine clinical settings.

Volatile Sulfur Compounds (VSC) - A family of gases present in human mouth air that result from the putrefactive activity of Gram-negative oral microorganisms on proteinaceous substrates present in saliva and dental deposits. Levels of VSC in periodontal pockets have been found to correlate with the severity of the disease process (Rizzo, 1967). The most common volatile sulfur compounds are: organic sulfur (S^-) dissolved sulfide (HS^-), hydrogen sulfide (H_2S), and methyl mercaptan (CH_3SH).

Bleeding on Probing (BOP) - Presence or absence of gingival bleeding observed during periodontal probing. Evaluated by placing the periodontal probe tip at the bottom of the pocket and moving it gently along the root surface.

Probing Depth (PD) - The distance in millimeters (mm) between the gingival margin and the clinical location of the periodontal probe tip against the epithelial attachment. Considered an indicator of periodontal health or disease activity.

Dental Plaque-Induced Gingivitis - An inflammatory process in the gingiva resulting from the interplay of bacterial plaque located at the gingival margin and host response.

Gingival Index (GI) - A data collection instrument used to assess the severity of gingival inflammation based on color, consistency, and bleeding on probing (Loe, 1967).

Gingival Crevicular Fluid (GCF) - An exudate that flows from the gingival sulcus or pocket and increases with inflammation. GCF is a medium for the product of VSC in the periodontal pockets.

Assumptions

The following assumptions were made:

1. The Diamond Probe/Perio 2000 is a valid and reliable device for measuring relative sulfide concentrations within periodontal pockets as adjunctive information to periodontal diagnosis and as a monitor of periodontal treatment efficiency.
2. BOP is a valid and reliable indicator for determining gingival health status (Greenstein, Caton & Polson, 1981; Greenstein, 1984; Kaldahl et al. 1990).
3. The GI is a valid and reliable instrument for measuring the gingival health status of individuals (Loe, 1967).
4. The examiner responsible for all data collection is a valid and reliable scorer as evidenced by test-retest data calibration.

5. Differences in gingival health are the result of the independent variables (hygiene versus non-hygiene) and not due to clinician contact during clinical procedures.

Limitations

The following limitations could affect the internal and external validity of this study:

1. Subjects were recruited from of the Old Dominion University campus community and from the Dental Hygiene Care Facility. The sample consisted of 39 medically healthy individuals, with clinically healthy gingiva. Findings can be generalized only to a similar population.
2. Subjects may have altered their home habits by decreasing or increasing their oral hygiene care.
3. Given that oral hygiene was withheld for 21 days, subjects may have deviated from the study protocol.
4. Subjects knowledge of participation in a research study may have affected their oral health behavior, and hence, the outcome of the study.
5. Keeping the examiner blind to treatment side minimized expectations of the examiner during scoring procedures.
6. Given the split-mouth design, transfer of bacteria across the mandibular arch could have occurred, confounding the measures obtained on the sides of the arch where hygiene care was continued.

Hypotheses

The following hypotheses were tested at the .05 level of significance:

1. There is no statistically significant correlation between sulfide levels and gingival inflammation.
2. There is no statistically significant correlation between sulfide levels and bleeding on probing.
3. There is no statistically significant difference in sulfide levels between the hygiene and non-hygiene sides over time.
4. There is no statistically significant difference in bleeding on probing between the hygiene and non-hygiene sides over time.
5. There is no statistically significant difference in gingival inflammation between hygiene and non-hygiene sides over time.

CHAPTER II

REVIEW OF THE LITERATURE

Periodontal disease is principally caused by Gram-negative, anaerobic bacterial infections. The review of literature that forms the theoretical foundation for this study focused on H_2S, its origin and characteristics, and its toxicity in periodontal pockets. CH_3SH, frequently associated with H_2S, was also reviewed. Toxic metabolites of bacterial origin are implicated as important destructive factors in periodontal disease. A change in the structure of the crevicular epithelial barrier permits an increase in accessibility of toxic metabolites to the underlying connective tissue layer, where they initiate a sequence of destructive inflammatory reactions (Ng & Tonzetich, 1984).

VSC are intermediate products from the bacterial putrefaction of proteins with sulphur-containing amino acids and proteins in the human oral cavity. Studies have demonstrated that H_2S and CH_3SH are predominant components of VSC (Solis-Graffar, Rustogi & Graffar, 1980; Persson, 1992; Persson, Claesson & Carlsson, 1989; Persson, Edlund, Claesson & Carlsson, 1990). Localized toxicity of H_2S and CH_3SH in the oral cavity has been studied (Rizzo, 1967; Johnson & Tonzetich, 1985; Johnson, Ng & Tonzetich, 1992; Johnson, Yaegaki & Tonzetich, 1992; Johnson, Yaegaki & Tonzetich, 1996; Ng & Tonzetich, 1984; Tonzetich & Lo, 1979). These researchers suggest that by-products of bacterial metabolism such as H_2S and CH_3SH are causative factors in the etiology of periodontal disease.

Since the increased concentrations of VSC in the oral cavity of periodontal patients correlate with the severity of periodontal involvement, VSC are believed to be toxic to oral tissue (Rizzo, 1967; Horowitz & Folke, 1972; Tonzetich & McBride, 1981;

Yaegaki & Sanada, 1992). However, one must keep in mind that a correlation does not indicate causation.

At 1 to 5 ppm in the air, H_2S is an extremely poisonous gas with a recognizable odor characteristic of rotten eggs (Grant & Schuman, 1993). H_2S produced in the gingival crevice has been analyzed to determine its role in human periodontal disease (Rizzo, 1967). Rizzo used filter paper strips impregnated with lead acetate to detect the H_2S production in the gingival crevice of periodontal patients. Observing an increased volume of VSC in the oral cavity of patients with periodontal disease, Rizzo concluded that VSC play an importance role in periodontal disease progression. According to Langendijk, Hagemann & Hoeven (1999), periodontal sulfate-reducing bacteria are associated with several clinical categories of periodontitis and with periodontal sites of increased pocket depth. Toxic sulfide products of these strictly anaerobic bacteria can accumulate in periodontal pockets in concentrations that may cause cellular destruction. Rizzo (1967) pointed out that H_2S is one of the most toxic metabolic by-products of anaerobic bacteria located in periodontal pockets. The frequencies of these anaerobic bacteria increase in periodontitis as compared with healthy sites in the oral cavity.

Ng and Tonzetich (1984) revealed that H_2S adversely affects protein synthesis of human gingival fibroblasts in culture. Upon exposure of collagen to elevated H_2S concentrations, the H_2S converted some acid-soluble collagen to a more soluble product, which could be extracted in neutral salt and analyzed by carboxymethyl cellulose chromatography. This effect on collagen solubility makes it more susceptible to enzymatic degradation and contributes to the increased destruction of collagen observed in thoil-treated fibroblast cultures (Johnson et al., 1992b).

Tonzetich and McBride (1981) observed differences in intermediate sulfide metabolism between non-pathogenic strains of *Bacteroides melaninogenicus var melaninogenicus* (CP-) and pathogenic *Bacteroides melaninogenicus* (CP+). The CP+ strains, which produced collagenase and protease and caused the formation of abscesses when injected subcutaneously into groins of guinea pigs, produced copious amounts of VSC, which consisted predominantly of CH_3SH. Although the CP-organisms did not grow as well as CP+, the differences in concentration of VSC may be only partly related to the disparity in growth rates. Results suggest that VSC analysis offer a convenient means of assessing strain differences and pathogenic potential of *Bacteroides melaninogenicus*. The addition of glucose to the medium depressed total volatile sulphur production by both CP+ and CP-strains, attributable mostly to lower H_2S levels.

Persson et al. (1989) reported that subgingival microbiotas recovered in deep periodontal pockets had a very high capacity to produce VSC in human serum. Researchers incubated bacterial samples from 7mm to 12mm periodontal pockets for seven days to determine the amount of VSC and the degradation of serum protein produced. Data suggest that H_2S was the predominant VSC in the serum, but significant amounts of CH_3SH were also formed. In addition, members of subgingival microbiotas had the capacity to degrade human serum proteins and form VSC compounds.

Persson (1992) later revealed that the most potent producers of H_2S were *Trepomena denticola* and black pigmented species, *Bacteriodes intermedius, Bacteroides loescheii, Porphyromonas endodontalis and Porphyromonas gingivalis. Porphyromonas gingivilis* also produced copious amounts of CH_3SH in serum protein.

Researchers have established a direct correlation between amounts of H_2S, CH_3SH, and severity of periodontal disease activity (Johnson et al., 1992a; Yaegaki & Sanada, 1992). By using radioactive, electrophoretic and chromatographic techniques, Tonzetich and Lo (1979) observed reaction of H_2S and saliva, collagen and gelatin. The exposure of oral mucosa to both H_2S and CH_3SH caused a marked increase in its permeability. The finding support the involvement of sulphur compounds in the etiology of periodontal disease. Since H_2S has been identified in periodontal pockets and is implicated in periodontal disease, results of the H_2S treatment of collagen and saliva could provide an insight into the possible mechanism of involvement in the disease process.

In summary, periodontal pockets are believed to be one of the main production sites of oral VSC. The generation of H_2S and CH_3SH could be due to the presence of the specific Gram-negative organisms in the gingival crevicular fluid or the effect of enzymes produced by organisms in the fluid. The clinical presentation of gingivitis is important to oral healthcare providers because of the association of gingivitis with poor oral hygiene and its association with the potential onset of plaque-induced gingivitis (Van Dyke et al., 1998). The value of VSC as an indicator of disease activity remains illusive.

CHAPTER III

METHODS AND MATERIALS

Research Design

A split-mouth design was used in an experimental gingivitis model to monitor the relationship between sulfide levels and two parameters of gingival health, BOP and GI, over a 21-day period. Using the experimental gingivitis model of Loe et al. (1965), reversible gingival inflammation was allowed to develop in subjects. This model allows researchers to evaluate subjects through the initiation and progression of plaque-induced gingivitis and to correlate sulfide levels, gingival inflammation, and bleeding on probing.

A split-mouth design allowed each subject to serve as his/her own control. In addition, randomization of mandibular quadrants to hygiene and non-hygiene sides increased the internal validity of the study. The two independent variables were daily oral hygiene care (H) on one side of the mandibular arch and non-hygiene care (NH) on the other side. The dependent variable measures were the Gingival Index (GI), bleeding on probing (BOP), and sulfide concentrations (SUL).

To optimize internal validity, a single calibrated examiner and the same equipment were used for all data collection. The Diamond Probe System check was performed at the completion of each patient to ensure the equipment was functioning properly.

At baseline, all subjects were given the same type of soft toothbrush and fluoride toothpaste[2] without any added ingredients for controlling gingivitis or calculus formation. No attempt was made to modify subjects' regular daily oral hygiene except on the NH

[2] Crest Regular Sodium Fluoride Anticavity Toothpaste. Active: Sodium Fluoride (0.15% w/fluoride ion).

side where, subjects were instructed not to brush or floss. Subjects were advised not to use mouthrinse for the duration of the study.

Sample Description and Selection

Subjects were recruited via flyers distributed throughout the Old Dominion University community and campus wide e-mail system. The final convenience sample consisted of 39 subjects, between age 19-62 years old, with healthy gingiva, who met the following inclusion criteria:

- 18 years of age or older.
- General good health, not pregnant.
- Free of orthodontic and prosthetic appliances.
- Minimum of 10 teeth in the mandibular arch.
- Gingival Index score of 0 to 1.
- Free of antibiotics for one-month prior to data collection.

Subjects with healthy gingiva ($GI \leq 1.0$) who agreed to participate in this study were enrolled. Each quadrant of the mandibular arch was randomly assigned to receive one of the two following treatments: (NH), withhold brushing and flossing on one side or (H), maintain current oral hygiene care.

Procedures, Materials, Data Collection Instrument

The Diamond Probe/Perio 2000 System®. A real-time, chairside system designed to evaluate relative Gram-negative bacteria in the gingival sulci and periodontal pockets with a silver sulfide probe sensor tip (Figure 1). When detecting sulfides, the system provides information on localized bacterial activity three ways: through a lighted display, by emitting an audible tone, and by providing quantitative sulfide levels.

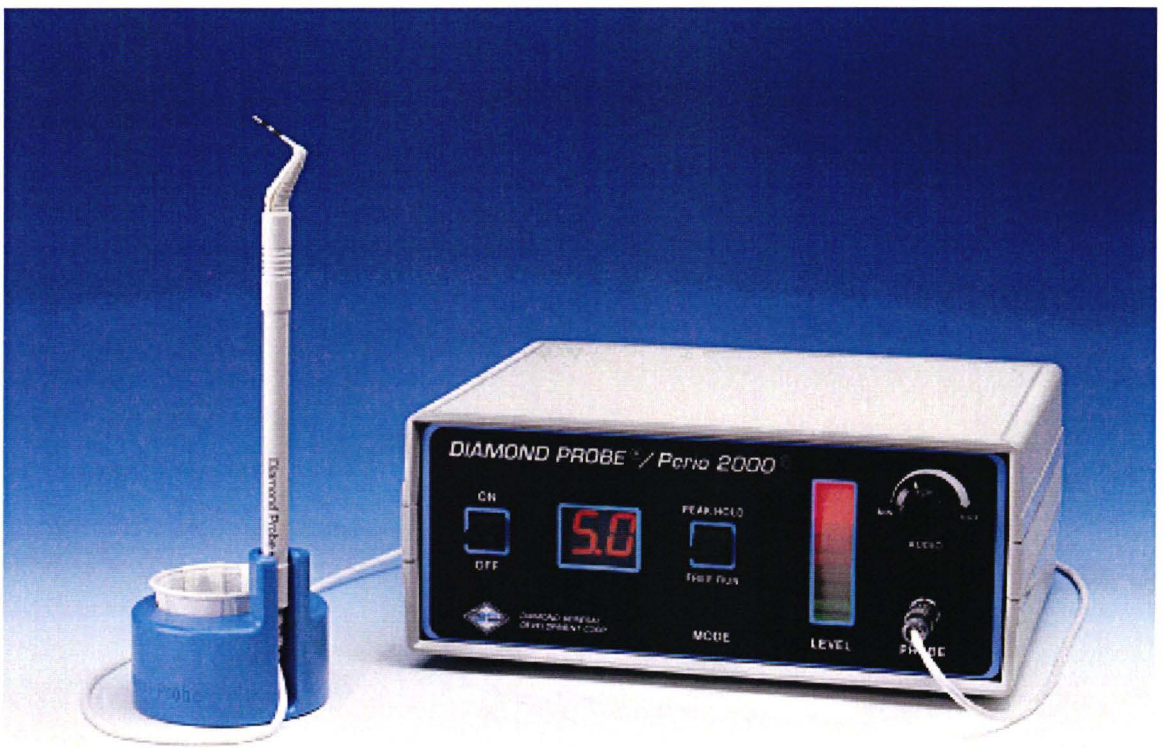

Figure 1. Research Model of Diamond Probe/Perio 2000 System® (Registered Trademark of Diamond General Development Corporation, Ann Arbor, MI).

Diamond Probe Sensor Tip®. The system incorporates a micro-sulfide sensor into a modified "Michigan O" style disposable periodontal probe (Diamond Probe Sensor Tip®) to measure probing depth, bleeding on probing, and sulfide levels (Figure 2). The various forms of sulfides (S^-, HS^-, H_2S, and CH_3SH) within the gingival crevicular fluid interact with the sensor tip of the probe to produce an electrochemical potential.

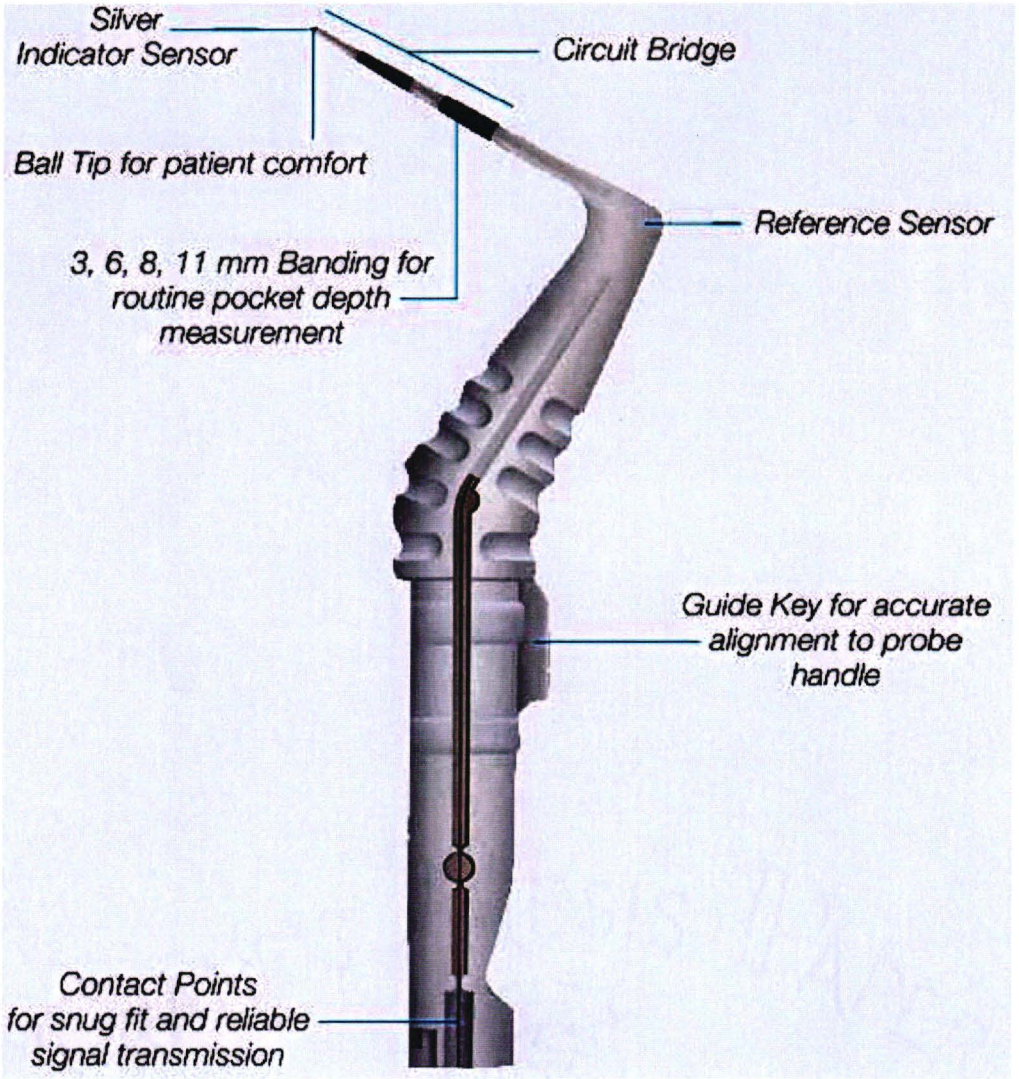

Figure 2. The Diamond Probe Sensor Tip® (Registered Trademark of Diamond General Development Corporation, Ann Arbor, MI).

According to the manufacturer, the value of the electrochemical potential is directly proportional to the concentration of sulfides, a direct measure of Gram-negative bacterial activity. The thin, rounded probe tip has incremental color-coded markings at 3, 6, 8, and 11 mm for easy measurement. The tip diameter of the Diamond Probe is 0.38 mm, which is the same diameter as the "Michigan O" probe (Figure 2). The probe handle connects the sensor tip with a handpiece cable to the system to capture sulfide levels. The Diamond Probe wash solution is distilled sterile water. The water is deionized and demineriliized to control minerals, which could hasten the sulfide ion, resulting in false negative readings. By using the wash solutions, false positives from mineral precipitants can be avoided. There is also an accessory stand for wash cup placement and temporary probe storage.

Diamond Probe System Check. A small amount of the compound aluminum sulfide (Al_2S_3), contained in a small vial, was used to confirm proper equipment operation. To secure the system check, the probe tip was first washed to ensure it was clean and moist. Then the probe tip was gently placed just above the center of the vial filter top. As the sulfide gas was emitted, a properly working unit will signal a reading within 20 seconds. The vial is recapped after the reading is obtained.

Sulfides detected by the sensor tip were transmitted via a handpiece cable connected to the Electronic Control Unit. The research model Control Unit displayed the sulfide concentration levels in three ways: digital, analog, and audio. The digital format displays a quantitative measurement of sulfides 0-10000 units (Table 1). The analog display provides a four-color light bar, which correlates with the digital format. The audio sound informs the clinician and patient that sulfides are present.

Equipment Preparation. To prepare the Diamond Probe/Perio 2000 System® for data collection, the following steps were used: 1. Hydrate and wash the probe tip. 2. Place a new disposable sterile probe sensor tip into the handle. 3. Turn the power switch to the ON position until Digital Display and Light Bar is illuminated. 4. Adjust the audio control to the midrange position. 5. Select Free Run mode. The Free Run mode was selected because the Digital Display responds in synchronization with actual sulfide concentrations being detected while Peak/Hold operational mode only remains the highest value recorded during each appointment.

Table 1. Possible Sulfide Range of the Diamond Probe/Perio 2000 System®

Digital Display	**Light Bar**	**Patient-Specific, Relative Sulfide Concentration**	**Sulfide Level**
0.0	Blank	< 0.5 unit	Low 0-1 unit
0.5	1st Green	0.5 unit	
1.0	2nd Green	1 unit	
1.5	1st Yellow	5 units	Moderate 5-10 units
2.0	2nd Yellow	10 units	
2.5	1st Orange	50 units	High 50-500 units
3.0	2nd Orange	100 units	
3.5	3rd Orange	500 units	
4.0	1st Red	1000 units	Very High 1000-10000 units
4.5	2nd Red	5000 units	
5.0	3rd Red	10000 units	

Tip Hydration. To hydrate the probe tip, two 5ml packages of wash solution were dispensed into a wash cup. The probe tip was immersed in the wash solution just beyond the first measurement band for at least 60 seconds, but no more than 5 minutes. During this time, the light-gray bands on the sensor tip begin to darken to alert the operator that tip has been properly hydrated and is ready to use.

Once thoroughly hydrated, the tip was gently agitated in the wash solution, without touching the sides or bottom of the wash cup. While agitating the tip, the foot switch was depressed until the first audible tones were heard. The first tone is a "bing" tone. At this point, the foot switch was released. The operator continued to agitate the probe tip for approximately three seconds until the second audible "bong" tone was heard. The probe tip was then removed from the wash solution. The Digital Display on the Control Unit read 0.0, which indicated that the tip is fully hydrated and ready to use.

Qualifying Appointment and Consent Procedures. The protocol was discussed with each subject. Subjects were screened to determine eligibility (Appendix A). If potential subjects met the inclusion criteria and agreed to participate, they were required to complete a comprehensive medical history (Appendix B). Once enrolled, subjects were given a thorough explanation of the study, its benefits and risks, and appointment procedures. Participants were asked to read the informed consent which was approved by the Institutional Board of Old Dominion University (Appendix C), and sign two copies, returning one copy to the principal investigator and keeping the other. Subjects were scheduled for subsequent data collection appointments. Screening procedures were completed in approximately 20 minutes.

Methodology. Each participant's mandibular arch was randomly assigned to one of two treatments: NH on one side and H on the other side. The Diamond Probe/Perio 2000 System® was used to measure PD, BOP, and SUL levels at baseline and three subsequent appointments (day 7, 14, and 21). Four sites on each mandibular tooth were examined (distofacial, facial, mesiofacial, and midlingual) for GI, BOP and SUL. Teeth numbers 17 and 32 were excluded from data collection. At the completion of the study, all subjects were followed to ensure their return to baseline gingival health status.

Instrumentation. To obtain GI measurements, the teeth and gingiva were thoroughly dried with compressed air. The tip of the probe was gently inserted to the base of the gingival sulcus and moved along the sulcus with light pressure to evaluate bleeding. Each of the four gingival areas was also evaluated for color change and assigned a score from 0 to 3 (Loe, 1967).

0= Normal gingival status

1= Mild inflammation-slightly change in color, slight edema. No bleeding on probing.

2= Moderate inflammation-redness, edema, and glazing. Bleeding on probing.

3= Severe inflammation-marked redness and edema, ulceration. Tendency to spontaneous bleeding.

BOP was also recorded as 0 (negative) or 1 (positive).

As the probe tip was gently moved throughout the sulcus, if SUL were detected an audible tone was heard and the light bar scaled up and down on the digital display. SUL levels were quantified in increments of 0.5. The numerical value shown was subsequently recorded.

Once SUL were detected, the probe tip was re-washed to remove residual sulfides; the system was reset for the next measurement. After completing the examination of the lingual or buccal sections of a quadrant, even without SUL or BOP present, the probe tip was re-washed. This procedure also was repeated when blood or plaque from the sulcus was visible on the probe tip.

Baseline Appointment. Before data collection, each subject's medical history was reviewed and updated. Baseline periodontal probing was performed on four sites (distofacial, facial, mesiofacial, and midlingual) of all mandibular teeth to determine periodontal health status. GI, BOP, and SUL were obtained at the same four sites (Appendix E).

Subsequent Appointments. Three subsequent data collection appointments were conducted over a 21-day period; GI, BOP, and SUL were collected at day 7, 14, and 21. Medical histories were reviewed and updated prior to any treatment.

Protection of Human Subjects

The protocol was submitted to the Old Dominion University Institutional Review Board for Protection of Human Subjects prior to study initiation. Approval was granted on April 2001. An extension was granted on November 20, 2001, and the project started on December 7, 2001.

1. **Potential Risks.** Potential risk from the project might be a consequence of reversible gingival inflammation. Subjects were asked to withhold brushing and flossing on one side of the mandibular arch for 21 days to allow dental plaque-induced gingivitis to occur. According to Loe et al. (1965), gingivitis is a reversible oral disease, which can be corrected with oral hygiene care that

includes daily brushing and flossing. At the conclusion of the study, subjects received thorough written and oral hygiene care instructions to reverse experimental gingivitis, which occurred during the study. Subjects were followed and observed until gingival health was restored to baseline status.

2. **Consent Procedures.** After participants received a thorough explanation of the study, procedures, and potential risks, each subject was asked to read and sign the informed consent in duplicate (Appendix C). Subject participation was voluntary, and they could withdraw at any time without fear of reprisal.

3. **Protection of Subjects Rights.** Confidentiality of all the participants was maintained by using code numbers throughout the study. Data collection forms identified subjects by code rather than name. Only the principal investigator maintained the subject code. All data were stored in a locked cabinet and will be destroyed three years after completion of the study.

4. **Potential benefits.** Subjects would benefit from knowing their periodontal health status (PD, GI, BOP, and SUL). Subjects would benefit from the thorough oral hygiene instructions provided at the completion of the study. The Diamond Probe/Perio 2000 System® may benefit the general population by detecting signs of plaque-induced gingival disease before bleeding on probing, gingival inflammation, or loss of clinical attachment.

5. **Risk-benefit ratio.** The adverse effect from creating experimental gingivitis is minimal. Subjects were provided with descriptive written and oral steps to reverse gingivitis after the study. The risk of an allergic reaction from the sulfide probe or hydrating solution accompanying the sulfide probe is very unlikely.

Statistical Analysis

All data were collected by a single trained, calibrated dental hygienist. Intra-rater reliability of the examiner was determined prior to study initiation by applying the paired t-test on six subjects: PD scores taken on two separate days. Based on the p-values of repeated measures on day 1 ($p = 0.0984$) and day 3 ($p = 0.1747$), there was no significant difference in scores. The examiner was reliable and proceeded to the data collection phase of the study.

Both descriptive and inferential statistical tests were used in data analysis because GI and SUL are intervally scaled and continuous in nature. BOP was nominally scaled and discrete in nature, but treated as ratio scaled data. Descriptive analyses included mean scores of GI, BOP, and SUL on both the H and NH sides; inferential tests included Pearson correlation and Wilcoxon matched-pairs signed rank. The Pearson correlation was used to establish the relationship between SUL and the periodontal parameters, BOP and GI. Given the small sample size, the Wilcoxon test was chosen to evaluate differences in GI, BOP, and SUL at four intervals between H and NH sides. A computerized statistical analysis system (SAS) was used and hypotheses were tested at the .05 level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

This study determined the capability of sulfides in detecting early dental plaque-induced gingivitis. Among the 39 subjects, 28 (71.79%) were women with a mean age of 24.35 (±3.82) and 11 (28.21%) were men with mean age of 29.00 (±12.17). The mean of age of the total group was 25.67 (±7.34). For each subject, half of the mandibular arch received daily hygiene care (H) and the other half received non-hygiene (NH) care for 21 days; a split-mouth design was utilized. GI, BOP, and SUL were recorded at each appointment. Based on the Central Limit Theorem, all the subsequent statistical analyses were done on average scores, using the mean scores on individual teeth as the observation (or data). Data were analyzed using the mean as the most common measure of central tendency, percentiles describing the relative position of a given score in relation to other scores, and the Pearson correlation test to determine the relationship between SUL and GI, SUL and BOP. Wilcoxon signed rank test was used to compare the differences in mean scores in GI, BOP, and SUL between H and NH sides.

Results

From the baseline to day 21, on the H side the mean GI scores increased from 0.15 to 0.29; BOP scores increased from 0.04 to 0.08, and SUL levels increased from 0.01 to 0.06. On the NH side, from baseline to day 21, mean GI sores increased from 0.10 to 1.04; BOP scores increased from 0.06 to 0.62, and SUL levels increased from 0.01 to 0.15 (Table 2). Mean scores of the GI, BOP and SUL levels on the NH side remained higher than on the H side from day 7 to day 21.

Table 2. Overall Mean Scores of GI, BOP and SUL on Hygiene Side (H) and Non-hygiene Side (NH)

Variable	Side	Intervals							
		Baseline		Day 7		Day 14		Day 21	
		Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
GI	H	0.15	0.21	0.17	0.21	0.23	0.26	0.29	0.47
	NH	0.10	0.16	0.53	0.31	0.71	0.27	1.04	0.48
SUL	H	0.01	0.02	0.01	0.02	0.03	0.03	0.06	0.06
	NH	0.01	0.02	0.02	0.02	0.05	0.05	0.15	0.08
BOP	H	0.04	0.06	0.05	0.08	0.10	0.14	0.08	0.10
	NH	0.06	0.10	0.11	0.14	0.19	0.19	0.62	1.34

N = 39

Mean GI scores on the NH side increased significantly from baseline to day 7, then continue to increase, peaking at day 21. Mean GI scores on the H side remained stable from baseline to day 21, lagging significantly behind NH side throughout the study (Figure 3).

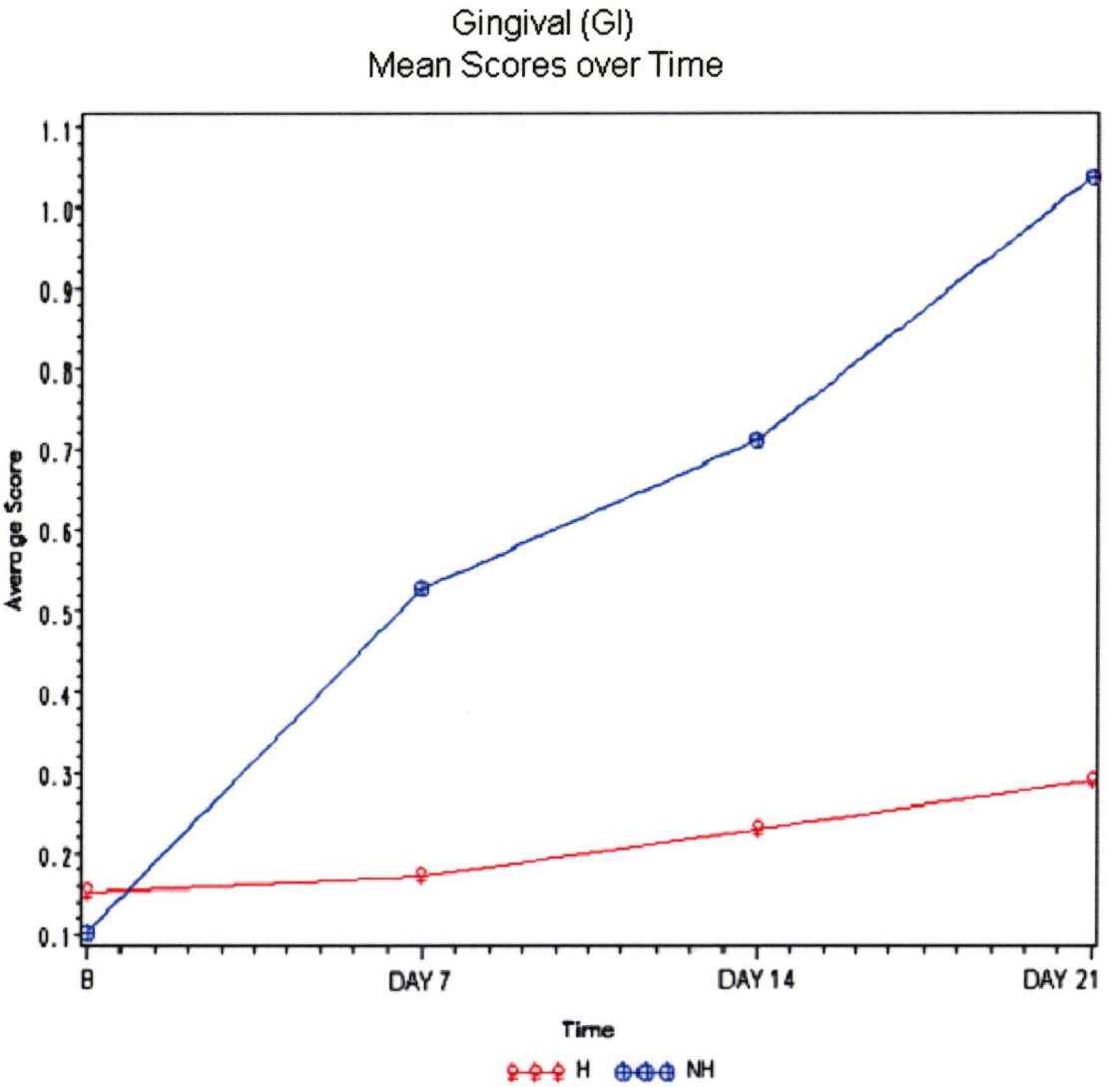

Figure 3. Mean GI Scores of Hygiene (H) and Non-Hygiene (NH) Sides Over Time

Mean BOP scores on the NH side steadily rose from baseline to day 14, then rapidly increased until day 21. Mean BOP scores on the H side reveal no significant changes between baseline and day 7, a slight increase from day 7 to day 14, then a slight decreased to day 21 (Figure 4).

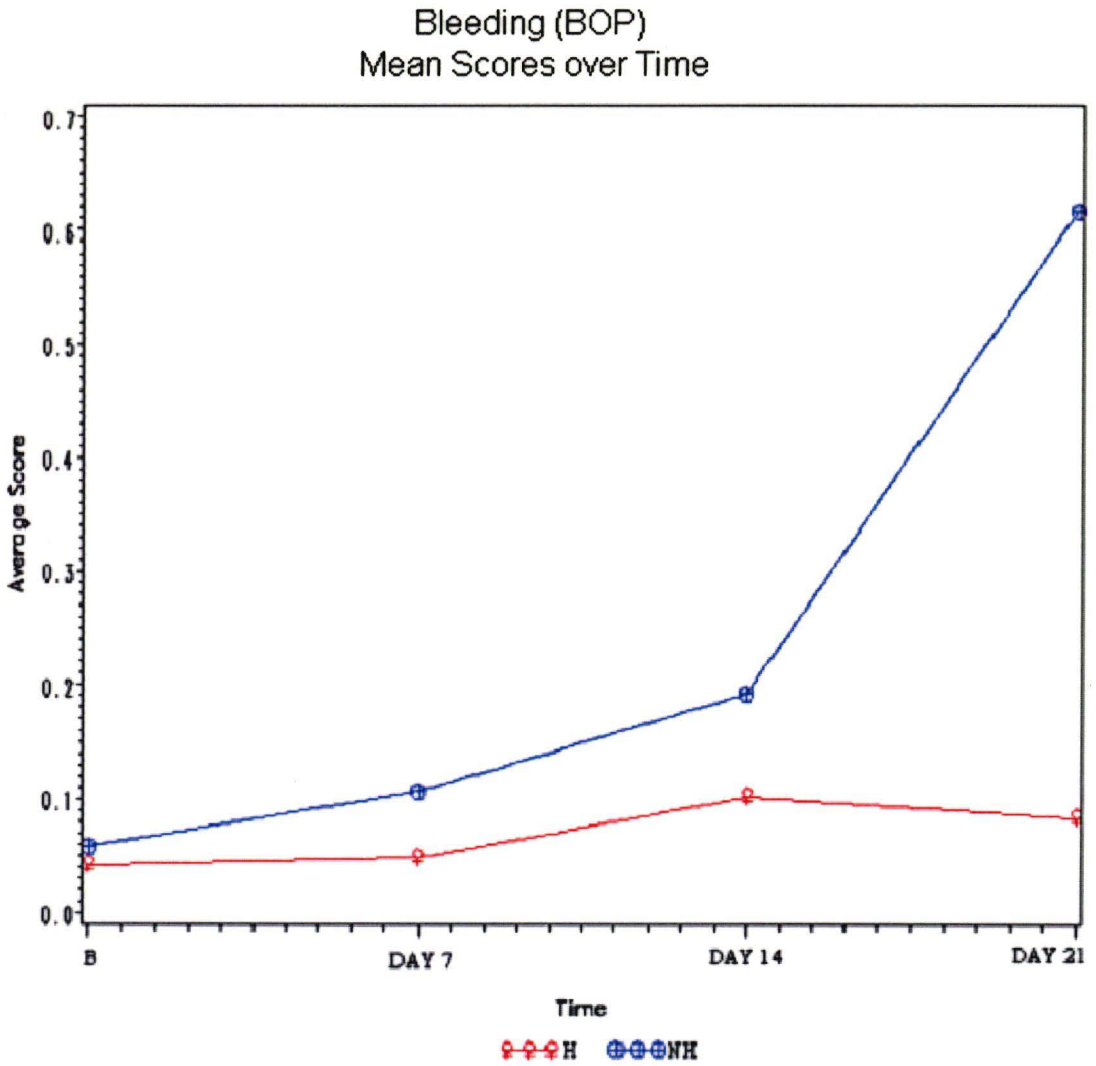

Figure 4. Mean BOP Scores of Hygiene (H) and Non-Hygiene (NH) Sides over Time

SUL on the NH side remained higher than on the H side throughout the study. Mean SUL on NH side, slowly increased from baseline until day 7, continued to rise until day 14, then rapidly increased until day 21. Mean SUL on H side remained steady from the baseline to day 7, slightly increased between day 7 and 14, then continued to increase until day 21, but lagged significantly behind scores on NH side throughout the study (Figure 5).

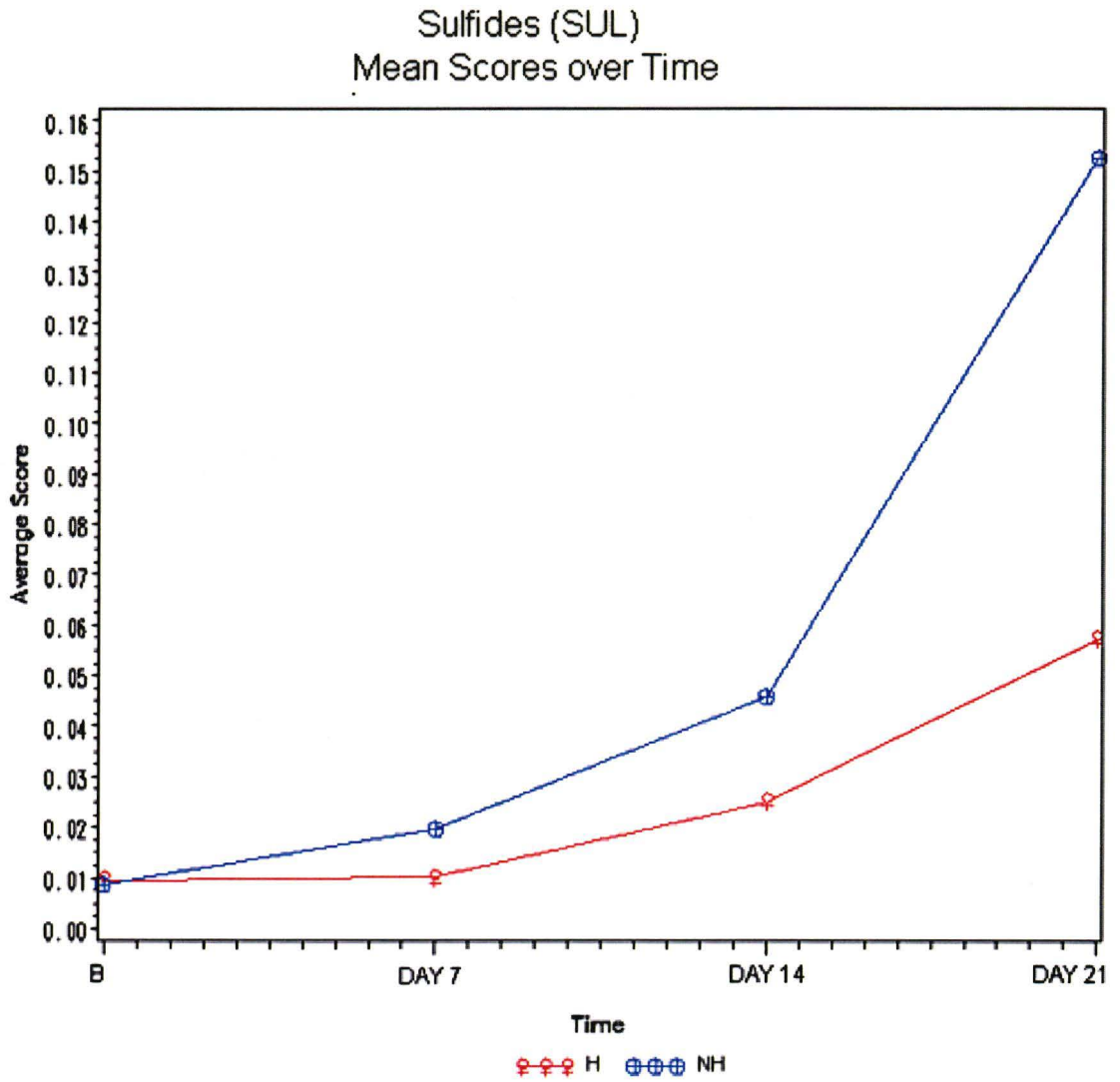

Figure 5. Mean SUL Scores of Hygiene (H) and Non-Hygiene (NH) Sides Over Time

Hypothesis 1. Data were analyzed to test the hypothesis that no significant correlation exists between SUL and GI. Statistics revealed a strong correlation between the GI and SUL for the NH side ($r = .66$, $p < .0001$) and moderate correlation for the H side ($r = .49$, $p < .0001$) (Table 3).

Table 3. Correlation Between Sulfide Levels and Gingival Index, and Sulfide Levels and Bleeding on Probing on Hygiene (H) and Non-Hygiene Side (NH)

	Pearson Correlation Coefficient r			
	Gingival Index		Bleeding on Probing	
Sulfide Levels	NH	H	NH	H
	r = .66	r = .49	r = .61	r = .45
	p< .0001*	p< .0001*	p< .0001*	p< .0001*

N= 39

* Indicates significance p= .05

Hypothesis 2. Data were analyzed to test the hypothesis that no significant correlation exists between SUL and BOP. Statistics revealed a strong correlation on the NH side was ($r = .61$, $p < .0001$) and moderate correlation on the H side ($r = .45$, $p < .0001$) (Table 3).

Hypothesis 3. Data were analyzed to determine if there was a statistically significant difference in GI between H and NH sides over time. Analysis was conducted

at four intervals to observe the differences in the GI between sides. Wilcoxon test results in Table 4 revealed significant difference in the GI between H and NH sides at day 7, 14 and 21 ($p < .0001$). Results suggest that with the progression of disease, the GI scores increased on the NH side.

Hypothesis 4. Data were analyzed to determine if there was a statistically significant difference in BOP between sides over time. Wilcoxon test results revealed a significant difference in BOP between the NH and H sides at day 7, 14, and 21 respectively ($p = .011$, $p = .006$, and $p < .0001$) (Table 4). Results suggest that with the progression of gingivitis, BOP scores increased on the NH side.

Hypothesis 5. Data were analyzed to determine if there was a statistically significant difference in SUL between the H and NH sides over time. Table 4 showed a significant difference between SUL on H and NH sides at day 7 ($p = .026$) and day 21 ($p < .0001$). However, at day 14 there was no significant difference between SUL on the H and NH sides ($p= .053$), revealing that SUL on the NH side were elevated at the end of week one and three, but not at the end of week two.

Table 4. Comparison of GI, BOP and SUL on Hygiene Side (H) with Non-hygiene Side (NH) Over Time.

Variable		Intervals			
		Baseline	Day 7	Day 14	Day 21
GI NH-H	d	-8.64	30.38	32.79	30.69
	p	.084	**< .0001**	**< .0001**	**<.0001**
BOP NH-H	d	1.59	12.64	13.92	32.59
	p	.743	**.011**	**.006**	**<.0001**
SUL NH-H	d	-1.18	10.46	9.72	27.62
	p	.785	**.026**	.053	**<.0001**

N= 39

p= .05

d –difference of mean scores between NH side and H side

p – calculated as 2-tailed test, bolded values indicate significance

Discussion

Hypothesis 1. Analysis of GI and SUL revealed a statistically significant positive correlation between SUL and GI on both H and NH sides, suggesting that SUL might be a useful marker for gingival disease activity. This finding supports the work of Solis-Graffar et al. (1980), who found a moderate correlation between the GCF and GI scores and a strong correlation between the GCF volume and H_2S production. The results of their research demonstrated that H_2S in GCF increases with the severity of gingival inflammation, therefore, SUL may be useful as an indicator of disease activity.

The increase of GI scores on the NH side at day 7, 14 and 21 is a direct gingival response to the continued bacterial plaque accumulation on the teeth. In addition, the significant increase of SUL on the NH side may be due to the proliferation of Gram-negative bacteria in plaque as documented by Loe et al. (1965). Since sulfide concentration is a metabolic by-product of proteolytic Gram-negative bacteria (Persson et al., 1990), increasing Gram-negative bacteria would increase sulphur by-product. Whether sulfur by-product is a contributor to the disease process, or merely a correlate remains unanswered.

GI scores on the H side did not change significantly until day 21 (Figure 3). During the study, subjects reported that they focused on their oral hygiene status and may have increased brushing and flossing time on the H side. Since a split-mouth design was used, microbes could have been transferred from one side to another. In addition, subjects, cognizant of the ever-growing plaque accumulation on the NH side, reported using their tongues to feel the difference between two sides, which may have facilitated transfer of bacteria from one side to another. The correlation between GI and SUL on the

H side supports that regular home care yields less gingival inflammation and lower sulfide levels.

Although this study demonstrated a positive correlation between the mean SUL and GI scores on both sides, the correlation was strong (r = .66) for NH side and moderate (r = .49) for H side (Table 3).

Hypothesis 2. Statistical analysis revealed a significant correlation between BOP and SUL on the NH sides as well as on the H side; however, the correlation was stronger on the NH side (r= .61) than on the H side (r = .45) (Table 3). Langendijk et al. (1999) suggests that presence of SUL in healthy subjects may be attributed to social-cultural differences of the patients, the aging of the plaque in the absence of oral hygiene procedures for 24 hours prior to data collection or absorption from the diet. This observation is supported by the work of Yaegaki and Sanada (1992) who found that VSC concentrations were higher in mouth air from patients with BOP than patients with no BOP. These findings suggest that SUL might be a useful indicator of gingival disease activity.

Hypothesis 3. Data revealed significant differences in GI over time between the H and NH sides at days 7, 14 and 21 (Table 4). Data support the assumption that the GI is a reliable clinical parameter for early-plaque induced gingivitis. Since a split-mouth design was used in this study, transferring bacteria from one side to another may have affected gingival scores. In contrast, findings failed to support the work of Van Dyke et al. (1998) that suggested that GI is remarkably insensitive as a quantitative tool of early pathogenesis.

Hypothesis 4. Data revealed a statistically significant difference in BOP over time between H and NH sides at days 7, 14 and 21 (Table 4). BOP becomes more severe as oral hygiene care is withheld. This finding supports BOP as an indicator of early clinical changes in the gingiva. By identifying sites containing SUL, in the absence of bleeding, represents new and valuable information to the clinician. Findings also complement the work of Greenstein et al. (1981) who first suggested the use of BOP, instead of visible inflammation, as an objective indicator of early gingival pathology.

Hypothesis 5. Data revealed a statistically significant difference in SUL over time between the H and NH side on day 7 and day 21 ($p = .026$ and $p =< .0001$ respectively), however, SUL concentrations at day 14 did not differ significantly (Table 4). This data supports the work of Zhou (2001), who found a statistical significant difference in SUL at days 5-6, 8, 12-14 and 15-16, 19, but not at day 10-12. Both studies suggest that SUL levels continue to rise throughout the study and peaked at the last data collection visit; however, there was one data collection where SUL concentrations were not significantly different between sides. Several explanations may be advanced for this phenomenon. One explanation is that there is a decline in anaerobic activity at approximately 10-14 days of the inflammation process where bacteria deplete their nutrients in the pocket. Another explanation may be related to host resistance. Perhaps around days 10-14, the strength of the host's immune system appears to resist the bacterial challenge.

Visual signs of inflammation are susceptible to subjective interpretation, can be masked by medications and smoking, and may not reflect the true periodontal status in areas inaccessible to visual inspection (Greenstein et al., 1981). Therefore, SUL scores in addition to BOP and GI may more accurately reflect the disease activity within the

pockets. This interpretation needs to be validated by studying the value of SUL scores in persons who use tobacco, or who take medications that can mask the clinical signs of inflammation.

It must be pointed out that data from this study suggest that SUL appear clinically at approximately the same time as BOP, but later than GI. Notably, by day 21, SUL levels almost tripled from baseline, suggesting that SUL increase with the progression of gingival inflammation. Additional studies are needed to support claims of SUL as a reliable indicator of early plaque-induced gingival disease. More research is needed to determine how SUL in the gingival sulci relate to ongoing gingivitis with the addition of microbial sampling. The evaluation of metabolic by-products of pathogenic bacteria may be one approach to diagnosing an active disease site.

CHAPTER V

SUMMARY AND CONCLUSIONS

Periodontal disease includes a group of inflammatory conditions of the periodontal tissue broadly categorized as gingivitis and periodontitis. Clinical evaluation of the periodontium traditionally consists of an assessment of gingiva, probing depth, loss of clinical attachment, bleeding on probing and radiographic interpretation of the periodontium. Therefore, reliable clinical parameters are necessary to predict early changes of gingival tissue. The ideal parameter should be valid, reliable, time efficient, and easily used by clinicians (Greenstein, 1984). In an attempt to develop objective measures, a variety of studies were undertaken based on the pathogenesis of periodontal disease (Persson et al, 1989; Kaldahl et al, 1990; Lang et al, 1986). The purpose of this study determined the relationship between VSC and gingival health status, and to identify the capability of VSC in detecting early plaque-induced gingivitis.

This study determined the short-term relationship between VSC and gingival health in subjects using a 21-day experimental gingivitis model. The Diamond Probe/Perio 2000 System® was used to obtain measurements of SUL concentration within gingival sulci of 39 subjects aged 19 to 62 years old, with healthy gingiva. All participants were volunteers and were free to withdraw at any time with no obligation. The periodontal status of each subject's mandibular arch was measured at four appointments with intervals of one week. Subjects withheld daily oral hygiene care from one randomly selected mandibular quadrant in order to allow experimental gingivitis to develop. Gingival health, BOP were measured with the GI (Loe, 1967) and SUL levels were quantified with the Diamond Probe/Perio 2000 System®. Upon completion of data

collection, all participants received verbal and written oral hygiene instructions, and were monitored until they returned to their baseline health status.

Findings from statistical analysis of mean GI and SUL scores revealed a statistically significant relationship between the GI and SUL on both the H and NH sides. Therefore, the null hypothesis that there is no statistically significant correlation between SUL and GI was rejected. There appears to be a strong positive relationship between GI and SUL scores. This finding suggests that use of SUL is a reliable indicator of plaque-induced gingivitis.

Findings from statistical analysis of mean BOP and SUL revealed a statistically significant relationship between BOP and SUL on both the H and NH sides. SUL correlate with progression of plaque-induced gingivitis. Therefore, the null hypothesis that there is no statistically significant relation between SUL and BOP was rejected. The use of BOP instead of GI as an indicator of early gingival pathology has an advantage of being a more objective indicator of disease activity (Greenstein et al., 1981). This finding implies that SUL may be a factor in progression of plaque-induced gingivitis, but this assumption needs further testing.

Statistical analysis revealed significant differences in GI between H and NH from baseline until the end of the study. Therefore, the null hypothesis that there is no statistically significant difference in GI between H and NH sides was rejected. According to Loe et al. (1965), in the course of experimental gingivitis, three out of 12 subjects developed gingivitis within 10 days, whereas nine subjects took between 15 to 21 days. The findings of this study confirm the research of Loe et al. (1965), which suggests that early gingival changes start occurring at day 7.

Findings from statistical analysis revealed significant differences in BOP over time between H and NH sides from day 7 until the end of the study. Therefore, the null hypothesis that there is no statistically significant difference in BOP over time between H and NH sides was rejected. This finding suggests that BOP is a valuable indicator of early changes in the gingiva and that BOP provides an objective diagnostic method for detecting the presence of inflammatory lesions clinically. These data support the results of Greentein et al. (1981) who first suggested the use of bleeding instead of visible inflammation as an objective, clinical indicator of early gingival pathology. Clinical findings from Lang et al. (1986) further indicate that bleeding after probing might be a more sensitive indicator for diagnosis of initial stages of gingivitis rather than clinical predictor for disease "activity" during periodontal maintenance.

Data suggest significant differences in SUL over time between H and NH sides from day 7 to day 21. Therefore, the null hypothesis that there is no statistically significant difference in SUL overtime between H and NH sides was rejected. SUL may be a useful marker in detecting early gingival disease activity. Overall, there was a high correlation of SUL and GI, and SUL and BOP scores on the NH side, and a moderate correlation of SUL and GI, and SUL and BOP for H side.

Given the findings, the following conclusions are made:

1. The Diamond Probe/Perio 2000 System® was able to detect sites with elevated levels of SUL, which can alert the dental professional to the presence of Gram-negative bacteria and the potential of disease activity.
2. SUL may be involved in the pathogenesis and progression of early plaque-induced gingivitis.

3. SUL may be a useful marker in detecting early plaque-induced gingivitis.

Considering the overall design and outcome of this study, the following recommendations are made:

1. Replicate the study using a larger sample to assure population validity and increase the likelihood of finding significant differences between SUL and GI.
2. Recruit a variety of participants (smokers, persons who are medically compromised or are taking certain medications which may mask gingival inflammation) to broaden the study population.
3. Conduct longitudinal studies to determine reliability of SUL in identifying active gingival and periodontal disease progression.
4. Replicate this study, but include microbial sampling to monitor the presence of Gram-negative anaerobic bacteria that may correlate with SUL.

Despite limitations of this study, the knowledge of potential sites of tissue-degrading activity will provide patients with immediate feedback so that treatment options for targeting bacteria may be considered. The continued investigation of the short and long-term relationship between SUL and periodontal disease activity may enable the profession to develop a new clinical technique for assessing active tissue breakdown. Such a technique would allow practitioners to intervene before tissue destruction has occurred. SUL analysis holds promise as one of these periodontal assessment techniques.

REFERENCES

American Academy of Periodontology. (1999). Informational Paper. The Pathogenesis of Periodontal Disease. *Journal of Periodontology*, 70(4), 457-470.

Fine, D. H., & Mandel, I. D. (1986). Indicators of periodontal disease activity: an evaluation. *Journal of Clinical Periodontology*, 13(5), 533-546.

Grant, W. M., & Schuman, J. S. (1993). Toxicology of the Eye: Effects on the Eyes and Visual System from Chemicals, Drugs, Metals and Minerals, Plants, Toxins, and Venoms; Also, Systemic s. Charles C Thomas Pub Ltd; Springfield, Ill., 4th edition: 797.

Greenstein, G. (1984). The role of bleeding upon probing in the diagnosis of periodontal disease. A literature review. *Journal of Periodontology*, 55(12), 684-688.

Greenstein, G., Caton, J. & Polson, M. (1981). Histologic characteristics associated with bleeding after probing and visual signs of inflammation. *Journal of Periodontology*, Aug; 52(8), 420-425.

Horowitz, A., & Folke, E.A. (1972). Hydrogen sulfide and periodontal disease. *Periodontal Abstracts*, XX (2) Summer, 59-62.

Johnson, P.W., & Tonzetich, J. (1985). Sulfur uptake by types I collagen from methyl mercaptan/dimethyl disulfide air mixtures. *Journal of Dental Research*, Dec; 64(12), 1361-1364.

Johnson, P., Yaegaki, K. & Tonzetich, J. (1996). Effect of methyl mercaptan on synthesis and degradation of collagen. *Journal of Periodontal Research*, Jul; 31(5), 323-329.

Johnson, P.W., Ng, W., & Tonzetich, J. (1992). Modulation of human gingival fibroblast cell metabolism by methyl mercaptan. *Journal of Periodontal Research*, Sep; 27(5), 476-483.

Johnson, P.W., Yaegaki, K. & Tonzetich, J. (1992). Effect of volatile thiol compounds on protein metabolism by human gingival fibroblasts. *Journal of Periodontal Research*, Nov; 27(6), 553-561.

Kaldahl,W., Kalkwarf, K., Patil, K. & Molver, M. (1990). Relationship of gingival bleeding, gingival suppuration, and gingival plaque attachment loss. *Journal of Periodontology*, 61(6), 347-351

Lang, N.P., Joss, A., Orsanic, T., Gusberti, F.A. & Siegrist, B.E. (1986). Bleeding on probing. A predictor for the progression of periodontal disease? *Journal of Clinical Periodontology*, Jul; 13(6), 590-596.

Langendijk, P.S., Hagemann, J., Hoeven, J.S (1999). Sulfate-reducing bacteria in periodontal pockets and in healthy oral sites. *Journal of Clinical Periodontology*, Sep; 26(9), 596-599.

Loe, H. (1967). The gingival index, the plaque index and the retention index system. *Journal of Periodontology*, 38(6), 610-616.

Loe, H., Jessen, J. & Theilade, E., (1965). Experimental gingivitis in man. *Journal of Periodontology*, 2(4), 177-187.

Morita, M., & Wang, HL. (2001). Relationship of Sulcular Sulfide Level to Severity of Periodontal Disease and BANA Test. *Journal of Periodontology*, Jan; 72(9), 74-78.

Ng, W., & Tonzetich, J. (1984). Effect of hydrogen sulfide and methyl mercaptan on the permeability of oral mucosa. *Journal of Dental Research*, Jul; 63(7), 994-997.

Page, R.C. (1985). Oral health status in the United States: prevalence of inflammatory periodontal diseases. *Journal of Dental Research*, 49(6), 354-367.

Persson, S. (1992). Hydrogen sulfide and methyl mercaptan in periodontal pockets. *Oral Microbiology Immunology*, Dec; 7(6), 378-379.

Persson, S., Claesson, R., & Carlsson, J. (1989). The capacity of subgingival microbiotas to produce volatile sulfur compounds in human serum. *Oral Microbiology Immunology*, 4(3), 169-172.

Persson, S., Edlund, M. B. Claesson, R. & Carlsson, J. (1990). The information of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiology Immunology*, 5(4), 195-201.

Rizzo, A. (1967). The possible role of hydrogen sulfide in human periodontal disease. Hydrogen sulfide production in periodontal pockets. *Periodontics*, 5 (5), 233-236.

Solis-Graffar, M. C., Rustogi, K.N. & Graffar, A. (1980). Hydrogen sulfide production from crevicular fluid. *Archs Oral Biology*, 51(10), 875-880.

Tonzetich, J. (1978). Oral malodor: an indicator of health status and oral cleanliness. *International Dental Journal*, 28(3), 309-319.

Tonzetich, J., & McBride, B. C. (1981). Characterization of volatile sulfur production by pathogenic and non-pathogenic strains of oral bacteroides. *Archs Oral Biology*, 26(12), 963-969.

Tonzetich, J., & Lo, K. (1979). Reaction of hydrogen sulphide with proteins associated with the human mouth. *Archs Oral Biology*, 23(10), 875-880.

Van Dyke, T.E., Offenbacher, S., Pihlstrom, B., Putt, M.S. & Trummel, C. (1998). What is gingivitis? Current understanding of prevention, treatment, measurement, pathogenesis and relation to periodontitis. *Journal of the International Academy of Periodontology*, 1(6), 5-15.

Yaegaki, K., & Sanada, K. (1992). Volatile sulfur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *Journal of Periodontal Research*, 27(4), 233-238.

Yaegaki, K., & Suetaka, T. (1989). Periodontal disease and precursors of oral malodorous components. *Journal of Dental Health*, 39(7), 733-741.

Zhou, H. (2001). Sulphur by-product: a potential indicator of early dental plaque-induced gingival activity. Unpublished thesis, Old Dominion University, Norfolk, Virginia.

Screening Consent Form

Sulfur By-Product: A Potential Indicator of Periodontal Disease Activity
Old Dominion University

Date _____ Examiner _____ Enroll subject: Yes( ) No ( )

Inclusion Criteria
Over 18 years of age
General good health
At least have 20 natural teeth
Gingival Index or 0 or 1

Exclusion Criteria
Diabetic condition
Communicable disease
Blood dyscrasias
Congenital heart disease or heart murmur
Hypertension
Pregnant
Antibiotic premedication
Antibiotics taken within one month
Rheumatic fever or rheumatic heart disease
Joint replacement within the last 2 years
Immune-suppressed
Gingival Index of 2 or greater

You are being asked to participate in a clinical trial, which involves detecting the sulfide levels subgingivally by using Diamond Probe/Perio 2000 System. Before you are examined, please accurately answer the following questions:

1. Are you pregnant? YES ( ) NO ( )
2. Do you have any heart conditions or trouble of any kind? YES ( ) NO ( )
   if you answer YES, please describe:_____
3. Have you ever had any joint replacements? YES ( ) When?_____ NO( )
4. Have you ever had rheumatic fever or rheumatic heart disease? YES( ) NO( )
5. Have you ever been told you need antibiotic pre-medication for dental treatment? YES( ) NO ( )
6. Are you allergic to any medications? YES ( ) NO ( )
7. Are you allergic to latex? YES ( ) NO ( )
8. Are you presently taking any drugs or medications? YES ( ) What? _____NO ( )
9. Have you ever had or have any of the following?
   - High blood pressure YES ( ) NO ( )
   - Tuberculosis YES ( ) NO ( )
   - HIV YES ( ) NO ( )
   - Blood disorder YES ( ) NO ( )
   - Abnormal bleeding YES ( ) NO ( )
   - Diabetes YES ( ) NO ( )

By signing below you agree to be examined to determine if you qualify for the study, you have answered the health questionnaire honestly and the screening process has been explained to you.

Client signature_____ Date_____

Examiner Signature_____ Date_____

MEDICAL ALERT

Name _____ Address _____ Phone _____ Physician _____ Address _____ Phone _____

Occupation _____ Address _____ Phone _____ Dentist _____ Address _____ Phone _____

Parent/Guar. _____ Address _____ Phone _____ Emerg. Contact _____ (relationship) Address _____ Phone _____

Date _____ Date of Birth _____ Social Security Number _____ Sex: M or F _____

G. W. HIRSCHFELD
SCHOOL OF DENTAL HYGIENE AND DENTAL ASSISTING
DENTAL HYGIENE CARE FACILITY

For safe, personalized dental hygiene care, a complete and accurate health history is necessary. Dental procedures may complicate or be complicated by existing conditions elsewhere in the body; general health factors influence response to treatment. Please give each question careful consideration. If your answer is YES to the question, put a circle around YES; if your answer is NO to the question, put a circle around NO. To specify condition, circle the appropriate condition. Answer all questions and fill in blank spaces when indicated. Answers to the following questions are for our records only and will be considered CONFIDENTIAL.

HEALTH HISTORY

1. How would you rate your health?
   Good Fair Poor
   a. Has there been any change in your general health within the past year? Yes No
   If yes, explain _____
2. My last physical examination was on _____
   a. LAB TESTS: _____ Results of Exam
   b. and TESTS: _____
3. Are you now receiving care from a Physician? Yes No
4. Who? _____
5. Have you been hospitalized or had a serious illness within the past five (5) years? Yes No
   a. If so, what was the problem? _____
6. Do you have or have you had any of the following?
   a. Rheumatic fever or rheumatic heart disease Yes No
   b. Congenital heart disease Yes No
   c. Heart trouble of any kind (heart murmur, heart attack, coronary insufficiency, coronary occlusion, angina, arteriosclerosis, stroke, pacemaker) Yes No
      1) Do you have chest pain when you exercise? Yes No
      2) Are you ever short of breath after mild exercise? Yes No
      3) Do your ankles swell? Yes No
      4) Do you get short of breath when you lie down? Yes No
   d. Cancer Yes No
   e. Allergy or hayfever Yes No
   f. Asthma or bronchitis Yes No
   g. Hives or a skin rash Yes No
   h. Fainting spells, seizures or epilepsy, headaches Yes No
   i. Diabetes Yes No
      1) Do you have to urinate (pass water) more than six times a day? Yes No
      2) Are you thirsty much of the time? Yes No
      3) Does your mouth frequently become dry? Yes No
      4) Weight gain or loss of more than 10 lbs.? Yes No
      5) Slow healing? Yes No
   j. Hepatitis, jaundice or liver disease Yes No
   k. Arthritis Yes No
   l. Inflammatory rheumatism (painful, swollen joints) Yes No
   m. Stomach ulcers Yes No
   n. Kidney trouble Yes No
   o. Tuberculosis - Positive TB or PPD Test?, Chest X ray? _____ Yes No
   p. Do you cough a lot or cough up blood? Yes No
   q. High blood pressure or low blood pressure Yes No
   r. Venereal disease - syphilis, gonorrhea, etc. Yes No
   s. Oral herpes/cold sores/fever blisters Yes No
   t. Mononucleosis Yes No
   u. Joint, hip, knee replacement/implants Yes No
   v. HIV Positive Yes No
   w. Nervous system disorders - cerebral palsy, etc. Yes No
   x. Emotional/ mental system disorders Yes No
7. Have you had abnormal or severe bleeding after tooth extractions, surgery, or injury? Yes No
   a. Do you bruise easily? Yes No
   b. Have you ever required a blood transfusion? Yes No
   If yes, explain the circumstances _____
8. Do you have any blood disorder? (bleeder, leukemia) Yes No
9. Do you have anemia? Yes No
10. Have you had surgery or x-ray treatment for a cancer, tumor, growth, or any condition? Yes No
11. Have you had medical x-rays in the last 5 years? Yes No
12. Are you taking any of the following?
   a. Antibiotics or sulfa drugs Yes No
   b. Anticoagulants (blood thinners) Yes No
   c. Medicine for high blood pressure Yes No
   d. Cortisone (steroids) Yes No
   e. Tranquilizers Yes No
   f. Aspirin Yes No
   g. Insulin, tolbutamide (Orinase) or similar drugs Yes No
   h. Digitalis or drugs for heart trouble Yes No
   i. Nitroglycerin Yes No
   j. Antihistamines Yes No
   k. Other _____
13. Are you taking any drug or medicine? Yes No
   If so, what?
14. Have you taken any of the above in the past six months? Yes No
   If so, why? _____
15. Are you allergic or have you reacted adversely to:
   a. Local anesthesia Yes No
   b. Penicillin or other antibiotics Yes No
   c. Sulfa drugs Yes No
   d. Barbiturates, sedatives, sleeping pills Yes No
   e. Aspirin Yes No
   f. Iodine Yes No
   g. Codeine/narcotics Yes No
   i. Latex Yes No
   h. Other _____
16. Are you employed in any situation which exposes you regularly to x-rays or other ionizing radiation? Yes No
17. Are you wearing contact lenses? Yes No
18. Do you have any disease, condition, or problem no listed above that you think I should know about? Yes No
   If so, please explain _____
19. Are you in recovery for alcoholism or substance abuse? Yes No

WOMEN SHOULD ANSWER THE FOLLOWING:

20. Are you pregnant? Yes No
   If yes, due date? _____

Comments on Positive Responses:

Contraindications for Treatment: (PDR Information)

Name/Dosage	Name/Dosage
PDR Page No.	PDR Page No.
Classification	Classification
Dental Consideration	Dental Consideration

Appendix C

Informed Consent Form of the
Old Dominion University

Informed Consent
Old Dominion University

INFORMED CONSENT DOCUMENT:
The purpose of this form is to give you information that may affect your decision whether to say YES or NO to participate in this training session, and to record the consent of those who say YES.

TITLE OF RESEARCH: Calibration Training for Sulphur By-Product: A Potential Indicator of Periodontal Disease Activity

RESEARCHERS: Principal Investigator Gayle McCombs, RDH MS, Michele Darby, RDH MS, and Alekaya (Alex) Pavolotskaya, RDH, BS, School of Dental Hygiene, College of Health Science, Old Dominion University, Norfolk, VA.

DESCRIPTION OF RESEARCH STUDY: The purpose of this training session is to train and standardize examiners involved in the conduct of clinical research. Training and calibration is needed in order to control or reduce examiner differences or variability in collecting measurements. The examiners will be calibrated against a "gold standard". Calibration consists of replicating assessment indices on patients with various levels of disease. A total of eight subjects will be recruited. Indices, which measure dental plaque, gingival inflammation, bleeding and pocket depth, will be repeated three times on six patients used in the actual training. Two patients will be chosen as "mock patients" before the actual training begins. Indices will be repeated on the mock patients twice.

If you decide to participate, you will join a group of 8 subjects. If you say YES, then your participation will require anywhere from 1 to 3 hours of your time. The decision of whether you will be a mock patient or training patient will depend on the status of your oral health and you will be made aware of which patient you will be before training begins. All training will be conducted at Dental Hygiene Care Facility at Old Dominion University.

EXCLUSIONARY CRITERIA: You should have completed a screening health history form. To the best of your knowledge, you should not have any medical condition that would keep you form participating in this study, such as (1) a need for antibiotic premedication prior to dental procedure or (2) other exclusionary medical complications listed on the screening health history form. You will need to have at least 20 natural teeth to be included in this session.

RISKS AND BENEFITS:

RISKS:

If you decide to participate in this study you may experience some mild discomfort to the gums from the repeated probings, but similar to what is felt at a regular dental examination. Dental probing is a procedure by which the dental professional measures the gum around your teeth by using a small dental instrument, which measures in millimeters. We will be repeating these measurements, but there is no obvious risk involved. You may experience some tenderness of your gums from the probings, but this will be minimal and should subside fairly quickly (few minutes to an hour).

By conducting the research in the Dental Hygiene Care Facility, the researchers tried to reduce the risks. All universal infection control and sterilization procedures will be followed. The researchers will provide careful instructions to all participants and phone numbers you can call if you have questions.

BENEFITS: The main benefit to you for participating in this study is that you will receive free periodontal screening. Other benefits include, depending on the amount of participation, $25.00 to 60.00. ($25.00 for one hour and $ 60.00 for 3 hours.)

COSTS AND PAYMENTS: The researchers want your decision about participating in this calibration to be absolutely voluntary, but you will receive anywhere from $25.00 to $60.00 at completion of the training for your participation. You will know in advance how much time will be required of you before you say YES.

NEW INFORMATION: If the researchers find new information during this session that would reasonable change your decision about participating, then they will give it to you.

CONFIDENTIALITY:
The researchers will take reasonable steps to keep private information, such as screening forms and medical histories confidential. The researchers will remove identifiers, such as names, from the case report forms and store information in a locked filing cabinet prior to it's processing. The results of this calibration may be used in reports, presentations, and publications; but the researchers will NOT identify you by name. Of course, your records may be subpoenaed by court order inspected by government bodies with oversight authority.

WITHDRAWAL PRIVILEDGE:
It is OK for you to say NO. Even you say YES now, you are free to say NO later, and walk away or withdraw from the study, at any time. Your decision will not affect any relationship you may have with Old Dominion University, nor cause a loss of benefit to which you might otherwise be entitled. The researchers reserve the right to withdraw your participation in this study, at any time, if they observe potential problems with your continued participation.

COMPENSATION FOR ILLNESS AND INJURY:
If you say YES, then your consent in this document does not waive your legal rights. However, in this event of harm arising from this study, Old Dominion University will not give you any money, insurance coverage, free medical care, or any other compensation for such injury. In the event you suffer injury as a result of participation in this research project, you may contact Gayle McCombs, Principal Investigator at 683-5150 or Dr. David Swain, Chair, Institutional Review Board, Old Dominion University at (757) 683-6028.

VOLUNTARY CONSENT:
By signing this form, you are saying several things. You are saying that you have read this form or have had it read to you, that you are satisfied that you understand this form, the research study, and its risks and benefits. The researchers should have answered any questions you may have had about the research. If you have any questions later on, then the researchers should be able to answer them: call Gayle McCombs, at 757-683-5150.

If at any time you feel pressured to participate, or if you have any further questions about your rights or this form, then you should call Dr. David Swain, Chair, Institutional Review Board, at 757-683-6028, or the Old Dominion University Office of Research, at 757-683-3460. And importantly, by signing below, you are telling the researchers YES that you agree to participate in this study. The researcher should give you a copy of this form for your records.

Subject's Name (Please Print) _____ Date _____

Subject's Signature_____
Witness's Signature_____ Date_____

INVESTIGATOR'S STATEMENT:
I certify that I have explained to this subject the nature and purpose of this research, including benefits, risks, costs, and any experimental procedures. I have described the rights and protections afforded to human subjects and have done nothing to pressure, coerce, or falsely entice this subject into participating. I am aware of my obligation under state and federal laws, and promise compliance. I have answered the subject's questions and have encouraged him/her to ask additional questions at any time during the course of this study. I have witnessed above signature(s) on this consent form.

Investigator's Signature_____ Date_____

Appendix D

Data Collection Forms for Gingival Index, Bleeding on Probing and Sulfide Level

SUBJECT _____ INITIALS _____

Gingival Index, Pocket Depth, Bleeding on Probing and Sulfide Level

1st Visit Date _____

Facial

	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M
GI																																							
SUL																																							
BOP																																							
PD																																							

	31			30			29			28			27			26			25			24			23			22			21			20			19			18		
GI																																									
SUL																																									
BOP																																									
PD																																									

Lingual

2nd Visit Date _____

Facial

	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M
GI																																								
SUL																																								
BOP																																								
PD																																								

	31			30			29			28			27			26			25			24			23			22			21			20			19			18		
GI																																								
SUL																																								
BOP																																								
PD																																								

Lingual

3rd Visit Date _____

	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M
Facial GI																																							
Facial SUL																																							
Facial BOP																																							
Facial PD																																							

	31			30			29			28			27			26			25			24			23			22			21			20			19			18		
Lingual GI																																									
Lingual SUL																																								
Lingual BOP																																								
Lingual PD																																								

4th Visit Date _____

	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M
Facial GI																																							
Facial SUL																																							
Facial BOP																																							
Facial PD																																							

	31			30			29			28			27			26			25			24			23			22			21			20			19			18		
Lingual GI																																									
Lingual SUL																																								
Lingual BOP																																								
Lingual PD																																								

5th Visit Date _____

	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M	D	F	M
Facial GI																																							
Facial SUL																																							
Facial BOP																																							
Facial PD																																							

	31			30			29			28			27			26			25			24			23			22			21			20			19			18		
Lingual GI																																									
Lingual SUL																																								
Lingual BOP																																								
Lingual PD																																								

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
1	N	1	2	0	2
1	B	1	6	0	1
1	N	2	28	0	1
1	B	2	6	0	1
1	N	3	35	0	1
1	B	3	7	0.5	0
1	N	4	49	4	19
1	B	4	0	1	5
2	N	1	0	0	1
2	B	1	2	0	2
2	N	2	7	0.5	1
2	B	2	2	0	2
2	N	3	14	1.5	6
2	B	3	11	0	1
2	N	4	18	2	5
2	B	4	3	1.5	1
3	N	1	0	0	0
3	B	1	0	0.5	0
3	N	2	8	0	0
3	B	2	0	0.5	0
3	N	3	8	0	0
3	B	3	2	2	2
3	N	4	10	0.5	0.5
3	B	4	2	0	0
4	N	1	6	0.5	3
4	B	1	5	0.5	1
4	N	2	12	0.5	8
4	B	2	5	0.5	1
4	N	3	24	2.5	11
4	B	3	9	0.5	6
4	N	4	36	7	25
4	B	4	28	1.5	1
5	N	1	0	0	0
5	B	1	5	0	0
5	N	2	11	0.5	4
5	B	2	5	0	0
5	N	3	15	1	11
5	B	3	0	0	2
5	N	4	54	3	17
5	B	4	0	0	0

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
6	N	1	3	0	0
6	B	1	3	0	0
6	N	2	13	0.5	3
6	B	2	5	0.5	1
6	N	3	26	1	13
6	B	3	5	1	7
6	N	4	55	5.5	18
6	B	4	0	2.5	3
7	N	1	1	1.5	0
7	B	1	3	0	0
7	N	2	15	0	2
7	B	2	3	0	0
7	N	3	16	3	7
7	B	3	1	1	6
7	N	4	28	7	21
7	B	4	0	0.5	7
8	N	1	10	2	15
8	B	1	6	1.5	8
8	N	2	47	2.5	20
8	B	2	14	0	12
8	N	3	31	8	28
8	B	3	12	1.5	19
8	N	4	62	13	245
8	B	4	51	5	11
9	N	1	3	0	5
9	B	1	0	1.5	4
9	N	2	28	0	2
9	B	2	10	1.5	4
9	N	3	22	2	8
9	B	3	6	2	7
9	N	4	26	3.5	7
9	B	4	13	4	4
10	N	1	1	1	0
10	B	1	5	0	0
10	N	2	6	0	0
10	B	2	5	0	0
10	N	3	10	0.5	7
10	B	3	6	0	1
10	N	4	11	2.5	5
10	B	4	5	1	0

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
11	N	1	0	0	0
11	B	1	1	0	0
11	N	2	5	1	0
11	B	2	1	0	0
11	N	3	8	0	0
11	B	3	2	0	0
11	N	4	25	5	5
11	B	4	0	0	0
12	N	1	0	0	0
12	B	1	5	0	0
12	N	2	2	0	0
12	B	2	5	0	0
12	N	3	12	2.5	4
12	B	3	0	0	1
12	N	4	14	4.5	6
12	B	4	0	0.5	0
13	N	1	2	1	2
13	B	1	5	0	3
13	N	2	26	1.5	7
13	B	2	8	1.5	1
13	N	3	26	1.5	7
13	B	3	8	1.5	1
13	N	4	27	4.5	17
13	B	4	1	3	4
14	N	1	0	0	1
14	B	1	8	0	3
14	N	2	5	0	2
14	B	2	8	0	3
14	N	3	15	0	5
14	B	3	6	0	4
14	N	4	34	3.5	5
14	B	4	1	0.5	0
15	N	1	0	0.5	1
15	B	1	3	0	0
15	N	2	19	0.5	2
15	B	2	2	0	0
15	N	3	22	0.5	2
15	B	3	0	0.5	1
15	N	4	27	2	11
15	B	4	1	0.5	2

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
16	N	1	0	0	0
16	B	1	0	0	1
16	N	2	15	0	6
16	B	2	0	0	1
16	N	3	25	1	10
16	B	3	11	0	2
16	N	4	28	5	21
16	B	4	3	0	1
17	N	1	5	0	2
17	B	1	6	0	2
17	N	2	17	0	3
17	B	2	6	0	3
17	N	3	17	0	7
17	B	3	13	0.5	2
17	N	4	34	4.5	9
17	B	4	22	4	3
18	N				
18	B				
18	N				
18	B				
18	N				
18	B				
18	N				
18	B				
19	N	1	22	0.5	5
19	B	1	34	0.5	5
19	N	2	27	0.5	8
19	B	2	34	0.5	5
19	N	3	30	1	12
19	B	3	20	0.5	9
19	N	4	42	4.5	9
19	B	4	30	3.5	4
20	N	1	0	0.5	0
20	B	1	0	0	0
20	N	2	18	1	1
20	B	2	4	0.5	0
20	N	3	24	2.5	0
20	B	3	5	1	0
20	N	4	25	4	9
20	B	4	21	6.5	6

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
21	N	1	1	0	0
21	B	1	2	0	4
21	N	2	15	0	4
21	B	2	2	0	1
21	N	3	20	0.5	3
21	B	3	0	0	1
21	N	4	23	1.5	9
21	B	4	3	1	0
22	N	1	0	0	0
22	B	1	5	0	0
22	N	2	3	0	0
22	B	2	5	0	0
22	N	3	22	0	0
22	B	3	4	0	1
22	N	4	30	6	11
22	B	4	4	1	0
23	N	1	0	0	0
23	B	1	1	0	0
23	N	2	7	0	0
23	B	2	1	0	0
23	N	3	20	0	1
23	B	3	2	0	0
23	N	4	22	3	15
23	B	4	0	0	0
24	N	1	0	0	0
24	B	1	2	0	0
24	N	2	16	0.5	0
24	B	2	2	0	0
24	N	3	16	0.5	7
24	B	3	2	0.5	2
24	N	4	24	1	8
24	B	4	2	2	7
25	N	1	0	0	2
25	B	1	0	0	2
25	N	2	7	0	2
25	B	2	0	0	2
25	N	3	16	1	4
25	B	3	7	1	2
25	N	4	20	3.5	17
25	B	4	0	0.5	0

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
26	N	1	3	0.5	2
26	B	1	4	1	1
26	N	2	8	1.5	3
26	B	2	1	1.5	1
26	N	3	18	2	0
26	B	3	6	2	1
26	N	4	18	7	7
26	B	4	1	2	2
27	N	1	0	0	2
27	B	1	0	0	0
27	N	2	12	0	2
27	B	2	0	0	0
27	N	3	24	0	3
27	B	3	4	0	0
27	N	4	23	4.5	16
27	B	4	3	0	0
28	N	1	6	0.5	4
28	B	1	5	0.5	1
28	N	2	16	1	9
28	B	2	3	0	2
28	N	3	28	3	4
28	B	3	21	2	0
28	N	4	28	5	13
28	B	4	13	4	9
29	N	1	0	0	1
29	B	1	0	1	2
29	N	2	7	0	1
29	B	2	3	0.5	2
29	N	3	10	1	1
29	B	3	4	0.5	3
29	N	4	6	1	4
29	B	4	2	0.5	2
30	N	1	3	0	1
30	B	1	2	0.5	0
30	N	2	19	0	3
30	B	2	2	0.5	0
30	N	3	20	1	2
30	B	3	0	2	3
30	N	4	27	3	23
30	B	4	0	1.5	0

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
31	N	1	3	0	0
31	B	1	0	0	0
31	N	2	14	0.5	2
31	B	2	0	0	0
31	N	3	11	0	1
31	B	3	0	0	1
31	N	4	31	4.5	6
31	B	4	2	0.5	0
32	N	1	1	0.5	0
32	B	1	0	0.5	1
32	N	2	14	1	0
32	B	2	2	0	1
32	N	3	18	2	8
32	B	3	2	0.5	0
32	N	4	9	4	12
32	B	4	5	2.5	5
33	N	1	14	0.5	9
33	B	1	15	0.5	2
33	N	2	19	1	9
33	B	2	11	1	3
33	N	3	28	2	12
33	B	3	37	1	12
33	N	4	56	2.5	17
33	B	4	56	2.5	2
34	N	1	0	0	0
34	B	1	2	0	0
34	N	2	15	1	0
34	B	2	2	0	0
34	N	3	20	1	4
34	B	3	2	0.5	3
34	N	4	27	3	6
34	B	4	6	0.5	0
35	N	1	2	0	0
35	B	1	9	0	0
35	N	2	26	0.5	0
35	B	2	9	0	0
35	N	3	42	2.5	8
35	B	3	6	1	0
35	N	4	50	7.5	7
35	B	4	0	1	1

SUBJECT	OH SIDE	VISIT	GI	SUL	BOP
36	N	1	7	0	1
36	B	1	6	1.5	2
36	N	2	12	1.5	2
36	B	2	3	1	6
36	N	3	13	1	2
36	B	3	7	2.5	3
36	N	4	14	7	16
36	B	4	14	2.5	6
37	N	1	6	0	1
37	B	1	6	0	0
37	N	2	11	0.5	3
37	B	2	6	0	0
37	N	3	13	0.5	1
37	B	3	4	0	0
37	N	4	34	2.5	1
37	B	4	15	1.5	3
38	N	1	10	0	3
38	B	1	11	0.5	1
38	N	2	24	2	2
38	B	2	11	1	1
38	N	3	13	0	1
38	B	3	17	1.5	6
38	N	4	36	6	14
38	B	4	7	3	1
39	N	1	0	0	0
39	B	1	0	0	0
39	N	2	11	0.5	0
39	B	2	0	0	0
39	N	3	21	0.5	6
39	B	3	3	0	0
39	N	4	27	3.5	4
39	B	4	3	0.5	0
40	N	1	0	0	0
40	B	1	0	0	0
40	N	2	13	1	3
40	B	2	0	0	0
40	N	3	23	3	3
40	B	3	0	0	1
40	N	4	21	5	12
40	B	4	0	0	1

VITAE

NAME: Aleksandra Pavolotskaya, BEd, RDH

EDUCATION:

2001-Present	Old Dominion University Gene W. Hirschfield School of Dental Hygiene Norfolk, Virginia Master of Science Degree Candidate
1993-1997	Indiana University School of Dentistry Indianapolis, Indiana Associate of Science in Dental Hygiene
1987-1991	Odessa Pedagogical Institute, Odessa, Ukraine Baccalaureate Degree in Elementary Education (*Graduated with Honors)*

LICENSURE:

August 2001	Virginia License No 0402202731
June 1997	Ohio License No 009410

PROFESSIONAL MEMBERSHIP:

1997-Present	American Dental Hygienists' Association
2002-Present	International Association for Dental Research

EXPERIENCE:

August 2001- June 2002	Graduate teaching assistant, Gene W. Hirschfield School of Dental Hygiene, Old Dominion University.
July 1991- August 1992	Elementary school teacher, Odessa School District, Ukraine. Experience in working with culturally diverse students. Didactic courses include Math, Russian, Ukrainian language, and Science in second and third grade.

RESEARCH:

January 2002- present	Currently finalizing thesis: *Sulfur By-Product: A Potential Indicator of Early Plaque-Induced Gingival Disease Activity* in partial fulfillment for Master of Science in Dental Hygiene degree requirements.