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Effectiveness of a Hydrogen Peroxide, Sodium Chloride, Sodium Bicarbonate Dentifrice Upon Microbiota Associated with Periodontitis

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EFFECTIVENESS OF A HYDROGEN PEROXIDE, SODIUM CHLORIDE, SODIUM BICARBONATE DENTIFRICE UPON MICROBIOTA ASSOCIATED WITH PERIODONTITIS

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ABSTRACT

EFFECTIVENESS OF A HYDROGEN PEROXIDE, SODIUM CHLORIDE, SODIUM BICARBONATE DENTIFRICE UPON MICROBIOTA ASSOCIATED WITH PERIODONTITIS

Jill M. Modi
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The purpose of this study was to investigate the effectiveness of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon quantitative changes in the sulcular microbial species Bacteroides melaninogenicus and in the gingival condition and plaque accumulation associated with periodontitis. Fifteen subjects from the Hampton Veteran's Administration Medical Center were selected according to a standard criteria. Split mouth experimental design was used. One arch of each patient was assigned randomly to periodontal instrumentation. Half the mouth then was assigned randomly an experimental or a control dentifrice to be utilized once daily. Culture analysis, the Gingival Index and the Plaque Index were performed initially to provide baseline data and at four and eight weeks. Intervals of two weeks provided review of home care procedures involved in the investigation. A two-way analysis of variance with alpha set at 0.05 was performed. Neither periodontal instrumentation nor home care regimen revealed a statistically significant difference.

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

CHAPTER 1

Introduction

Periodontal disease involves the breakdown of tissues that surround and support the teeth and currently is existent in the majority of the dentulous population.[72] Current therapy for periodontal disease entails a variety of treatment modalities, both surgical and nonsurgical. Nonsurgical procedures presently have not been validated through long-term research techniques. Generally, the nonsurgical methods have been directed toward bacterial plaque reduction, which potentially may lead to a decreased inflammatory condition. Several bacterial pathogens, including gram negative motile anaerobic bacteria, have been associated with the presence of periodontal inflammation.[4,16,18,33,41,42,75,82,84,99] A method of determining the presence of these sulcular bacterial complexes exists through culture media analysis.[35,57,81,85,89] Components of the experimental dentifrice of hydrogen peroxide, sodium chloride, sodium bicarbonate have been implemented in home care regimens in single or combined forms.[9,30,32,34,61,78,79,110,112] Performance of the dentifrice with and without scaling and root planing instrumentation lacks long-term investigation results. The effects of the presence and absence of scaling and root planing with use of the

assigned dentifrice upon the microbial status have not been documented in previously reviewed literature. The use of these procedures may or may not provide benefits when utilized to decrease periodontal inflammation.

This investigation dealt with a nonsurgical treatment modality involving the use of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice. Effects of the dentifrice were studied upon dentition that had and had not received periodontal instrumentation. Effectiveness of the home care regimen was performed through culture media analysis, the Gingival Index, and the Plaque Index.

Statement of the Problem

This study addressed the following questions:

1. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon anaerobic sulcular microbiota, Bacteroides melaninogenicus, with exposure to periodontal instrumentation?

2. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon anaerobic sulcular microbiota, Bacteroides melaninogenicus, without exposure to periodontal instrumentation?

3. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon gingival inflammation of subjects with exposure to periodontal instrumentation?

4. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon gin-

gival inflammation of subjects without exposure to periodontal instrumentation?

5. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon plaque accumulation of subjects with exposure to periodontal instrumentation?

6. What are the effects of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice upon plaque accumulation of subjects without exposure to periodontal instrumentation?

Significance of the Problem

A total of 95 percent of the dentulous population in the United States has been diagnosed as having periodontal disease.[72] Current therapy for periodontal disease includes a variety of surgical and nonsurgical treatment modalities. Various modalities are required for different treatment needs, such as pocket elimination and root surface debridement. One aspect of a nonsurgical treatment regimen, which was investigated in this research, included monitoring microbiota to observe the disease process and treatment effects.

The pathogenesis of periodontal disease has been proposedly associated to related bacterial complexes.[4, 8, 13, 18,32,34,35,40-42,50,57,58,61,67,74,75,80,82,85,89,108] The relationship of certain microbial complexes to various stages of periodontal disease is the rationale underlying microbial sampling as a method of monitoring periodontal

disease.[29,30,33,34] Culture media provides a macroscopic method for determining presence or absence of various microbial species obtained through sampling. Analysis and study of microbiota have been performed through plated culture media by many past and present investigators.[35,57,81,85,89]

Utilization of the chemotherapeutic agents, three percent hydrogen peroxide, sodium chloride, and sodium bicarbonate, has been proposed as a nonsurgical treatment measure.[29, 30,33,34] Chemotherapeutic usage is founded by the principle of the bacterial etiology of periodontal disease. If the causative factors of the disease process are removed, the destructive potential of the disease itself will, in turn, be removed.

Scaling and root planing have been investigated largely as methods of deposit removal.[5,7,25,26,43,70,87,94,97,103] A comparison of the effects of scaling and root planing versus no scaling and root planing on sulcular microbiota, in addition to use of the experimental dentifrice, has not been documented previously. This investigation may contribute to this minimally supported area.

Through implementation of this investigation, several concepts were examined which may provide additional knowledge to the current diagnosis and treatment modalities of periodontal disease. First, the use of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice, as compared to a distilled water, sodium bicarbonate dentifrice, in the

treatment of mild to moderate periodontal disease may provide information concerning nonsurgical periodontal treatment modalities. Second, the comparison of effects of the assigned dentifrice upon dentition which has and has not received periodontal instrumentation may supply evidence for the usage or nonusage of instrumentation prior to periodontal therapy. Periodontal disease is a major contributor toward the prevalence of dental disease; however, additional study needs to be performed concerning causation, diagnosis, and treatment. This investigation will provide additional information concerning these areas.

Definition of Terms

The following terms were defined for use in this investigation:

American Society of Anesthesiologists (A.S.A.) Category I (medical risk)--A normal healthy patient. No treatment modifications other than stress reduction (if indicated) was required.[54] Any patient classified in this medical risk category was a possible participant for the investigation.

American Society of Anesthesiologists (A.S.A.) Category II (medical risk)--A patient with mild to moderate systemic disease. Treatment modifications and stress reduction may have been indicated.[54] Any patient classified in this medical risk category was a possible participant for the investigation.

Control teeth--Two of the following four teeth: first premolar, second premolar, first molar, second molar were

examined for data collection. If all four teeth were present, the second premolar and first molar were referred to as the control teeth and used for periodic evaluation. These teeth were included in the two unilateral quadrants of the oral cavity that received the control dentifrice of distilled water and sodium bicarbonate.

Cultural bacterial measurement--Evaluation of Bacteroides melaninogenicus, a microbial species associated with periodontitis, through the use of plated culture media. A plaque sample of sulcular microbiota was removed at zero, four, and eight weeks from established pocket areas and evaluated for content on plated culture media. The microbial status of the Bacteroides species was recorded quantitatively.

Examined sulcular microbiota--Anaerobic Bacteroides melaninagenicus species located in the gingival sulcus was examined.

Experimental teeth--Two of the following four teeth: first premolar, second premolar, first molar, second molar were used for data collection. If all four of these teeth were present, the second premolar and first molar were referred to as the experimental teeth, and used for periodic evaluation. These teeth were included in the two unilateral quadrants of the oral cavity that received the experimental dentifrice of hydrogen peroxide, sodium chloride, and sodium bicarbonate.

Flossing--A 12-to 15-inch length of unwaxed floss was

used. The patient wrapped the two ends of the floss around the two middle fingers until approximately four inches of floss remained between the two fingers. The thumb and index fingers of both hands were used to guide the floss in a controlled manner. The floss was slowly directed interproximally through use of a back and forth motion. Once the floss was interproximal next to the gingival margin, the ends were formed into a "c" shape about one side of one tooth. An up and down scraping motion was then used to remove interproximal plaque and to distribute the dentifrice interproximally. The floss was then lifted up and over the interdental papilla, and the same procedure followed for the adjacent tooth. All interproximal surfaces and distal surfaces of the last posterior teeth were flossed.[111]

Gingival condition--The state of health possessed by the gingival tissues as determined by the Gingival Index.

Gingival Index--The index system used in the investigation to assess the condition of gingival health. Gingival tissues surrounding experimental and control teeth were given a score of 1, 2, or 3 for four gingival areas including distobuccal, buccal, mesiobuccal, and lingual, respectfully. Criteria included the following: 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation.[45] These criteria represent a modification of the original index.

Hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice--1/2 teaspoon 3% hydrogen peroxide, 1/2 teaspoon

distilled water, 1/4 teaspoon sodium chloride, and 3 1/2 teaspoons sodium bicarbonate combined to form a soft paste. The dentifrice was incorporated into the gingival sulcus through use of a fine rubber tip stimulator and sulcular toothbrush utilizing a sulcular brushing technique. Dental floss was used following the brushing procedure.

Patient home care regimen--The patient used unwaxed floss, a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice, and a sodium bicarbonate, distilled water dentifrice once daily. The dentifrice was incorporated into two unilateral quadrants through use of a fine rubber tip stimulator and a two-row multi-tufted nylon bristled sulcus toothbrush. The patient first distributed the dentifrice of sodium bicarbonate, distilled water along the gingival margins of teeth located on one side of the mouth, and incorporated the paste into the gingival sulcus utilizing a fine rubber tip stimulator, followed by a sulcus toothbrush. A sulcular brushing technique was used, followed by flossing of the completed side. This procedure was followed for the two opposing unilateral quadrants, utilizing the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice. The patient rinsed thoroughly with water after each two quadrants had been completed.

Periodontal instrumentation--Oral prophylaxis which consisted of scaling, root planing, and polishing procedures upon the dentition for the purpose of complete calculus deposit removal. Complete deposit removal was determined

through exploration of smooth tooth and root surfaces. Instrumentation was performed on either the maxillary or mandibular arch, which was determined through a random selection procedure.

Periodontal tissues--Gingival and sulcular tissue portions located in the oral cavity.

Periodontitis--For the purpose of this study, periodontitis was defined as an inflammatory condition of the periodontium involving 4-7 mm pocket depths and gingival inflammation ranging from mild to severe.[19]

Plaque accumulation--The severity and location of plaque deposits as determined by the Plaque Index.

Plaque Index--The index system used in this investigation to assess the accumulation of soft debris. Experimental and control teeth were given a score of 1, 2, or 3 for accumulation of soft debris aggregates found on buccal and lingual surfaces, respectively. Criteria included the following: 1 = light accumulation; 2 = moderate accumulation; 3 = abundant accumulaion.[45] These criteria represent a modification of the original index.

Pocket depth--The measurement of the pocket from the junctional epithelial attachment to the gingival margin as determined through the use of a color-coded probe with millimeter markings of 3-6-9-12.

Pocket depth measurement--The color-coded probe with millimeter markings of 3-6-9-12 was inserted beneath the gingival margin, with the probe parallel with the long axis

of the tooth. The tip of the probe was walked along the bottom of the sulcus and a total of six measurements were recorded for each tooth: disto-buccal beneath the contact area, buccal, mesio-buccal beneath the contact area, disto-lingual beneath the contact area, lingual, and mesio-lingual beneath the contact area.[68]

Posterior quadrant--Free gingival margin, attached gingiva, gingival sulcus, and tooth numbers 1-5, 12-16, 17-21, and 28-32 of the dentition located in the premolar/molar area.

Preventive Therapist--An individual who was responsible for instruction of each subject in the following oral hygiene procedures: (1) mixing of the prescribed dentifrice; (2) distribution of the dentifrice on the gingival margin; (3) modified Bass toothbrushing technique; (4) use of the rubber-tip stimulator; and (5) flossing.

Root planing--The process by which the surfaces of the roots were made smooth by the removal of residual fine calculus and diseased cementum through the use of instrumentation.[111] Instruments used for the procedure included Gracey curets (1/2, 7/8, 11/12, 13/14) and McCall curet (19/20).

Rubber tip stimulator utilization--Placement of the stimulator tip at a 45^{o} angle gently below the gingival margin. The procedure began at the distal portion of the first tooth in the designated quadrant and the tip was slowly vibrated around each tooth in the quadrant.[79] A separate rubber tip

was used for each treatment procedure.

Scaling--The procedure by which calculus was removed from the surfaces of the teeth through the use of instrumentation.[111] Instruments used for the procedure included Gracey curets (1/2, 7/8, 11/12, 13/14), Jacquette scalers (H5/33, 14/15), McCall curet (19/20), and Orban Files (10/11, 12/13).

Sodium bicarbonate, distilled water dentifrice--Three and one-half teaspoons sodium bicarbonate and one teaspoon distilled water were combined to form a soft paste. The dentifrice was then incorporated into the gingival sulcus through use of a fine rubber tip stimulator and sulcular toothbrush through use of a sulcular brushing technique. Dental floss was used following the brushing procedure.

Sulcular brushing technique (Modified Bass Method)--The placement of the toothbrush bristles at a 45^{o} angle toward the cervical third of the tooth, filament tips placed gently into the gingival sulcus, and a vibratory stroke used for the purpose of plaque removal and induction of dentifrice. The brush was rolled over the tooth crown toward the occlusal surface following the vibratory stroke.[111] A separate toothbrush was used for each treatment procedure.

Assumptions

For the purposes of this investigation, the following assumptions were made:

1. Subjects performed the two assigned regimens once daily upon the entire dentition as instructed.

2. Subjects understood instructions for the prescribed home care regimen as presented by the preventive therapist.

Limitations

The following limitations may have threatened the validity of this investigation:

1. Internal validity factors of history and maturation may have occurred due to lack of separate experimental and control groups.

2. Subject selection bias may have been present due to the use of an intact group from the Hampton Veteran's Administration Medical Center Dental Clinic. This bias was controlled through random assignment of unilateral quadrants to experimental and control sides for treatment modalities.

3. Subject mortality occurred; however, this limitation was partially controlled through utilization of a short-term investigation.

4. The Hawthorne effect may have occurred due to the subjects' knowledge of participation in the study.

5. The results of this study can only be generalized to similar populations due to the method of subject selection for this investigation.

6. The experimenter effect may have occurred due to close contact of the subjects with the experimenter.

7. Exposure of the subjects to a new oral hygiene regimen may have produced a novelty effect, which may have

caused a perception of the treatment newness as beneficial.

Hypotheses

H_{0_1}: There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice and scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice and scaling and root planing as measured by plated culture media.

H_{0_2}: There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides melaniogenicus in patients treated with a hydrogen peroxide, sodium cloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media.

H_{0_3}: There is no statistically significant interaction at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by plated culture media.

H_{0_4}: There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides

melaninogenicus in patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media.

H_{0_5}: There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium cloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice with scaling and root planing as measured by the Gingival Index.

H_{0_6}: There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium cloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index.

H_{0_7}: There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Gingival Index.

H_{0_8}: There is no statistically significant difference

at the 0.05 level in the gingival condition of patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index.

H_{0_9}: There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate distilled water dentifrice with scaling and root planing as measured by the Plaque Index.

$H_{0_{10}}$: There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index.

$H_{0_{11}}$: There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Plaque Index.

$H_{O_{12}}$: There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index.

Methodology

Fifteen male and female subjects between the ages of 20 to 70 years were selected for inclusion in this study. Each subject had a minimum of twenty teeth and a current status of periodontal inflammation that involved experimental teeth with 4-7 mm pocket depths. Two unilateral quadrants of each subject were assigned randomly as one of two sides, experimental or control. Once daily the experimental side received the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice in the prescribed home care regimen; while the control side received the distilled water, sodium bicarbonate dentifrice. Randomly assigned maxillary or mandibular arches were scaled and root planed in every subject at the initial appointment. Oral hygiene instructions were presented to each subject by a preventive therapist at the initial appointment. Instructions were reviewed at appointments in two-week intervals (week six being optional) to insure patient compliance.

The research was conducted in the dental clinic located at the Hampton Veteran's Administration Medical

Center. One principal investigator who also served as the dental hygienist and one preventive therapist were involved. Anaerobic microbial analysis through use of plated culture media, Gingival Index, and Plaque Index were monitored initially and at four-week intervals over an eight-week period (a total of three appointments). Initial screening of patients for acceptability in the study was performed by the principal investigator and staff dentists of the Hampton Veteran's Administration Medical Center Dental Clinic. A two-way analysis of variance test was utilized in conjunction with the Statistical Analysis System Software Program (also referred to as SAS).

CHAPTER 2

Review of The Literature

Periodontal disease involves the breakdown of tissues that surround and support the teeth and currently is existent in 95% of the dentulous population.[72] Current therapy for periodontal disease entails a variety of treatment modalities, both surgical and nonsurgical. This literature review pertains to several aspects of nonsurgical treatment forms. Nonsurgical modalities associated with this review include scaling and root planing; use of a 3% hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice; and relevant home care techniques.

Literature relevant to this investigation has been reviewed and discussed in the following sections: (1) microbiota associated with periodontal disease and corresponding evaluation; (2) the microbial species Bacteroides melaninogenicus, its relationship with periodontal disease, and its detection through plated culture media; (3) Löe's Gingival Index and Plaque Index systems for assessment of the gingival condition[45]; (4) home care instruction effectiveness and the proficiency of scaling and root planing in the removal of calcified bacterial deposits; (5) the specific plaque hypothesis in relation to periodontal disease treatment and occurrence; and (6) three percent

hydrogen peroxide, sodium chloride, and sodium bicarbonate as chemotherapeutic agents in the treatment of periodontal disease.

Microbiota Associated with Periodontal Disease and Corresponding Evaluation

Previous studies that have investigated microbial compositions of healthy and diseased periodontal tissue indicate explicit content differences when samples are obtained from the same individual.[4,10,13,18,32,34,40,42,50,58,61,67,74,75,80,82,109] The active state of periodontal disease has been shown to consist of oral microbiota that differ both quantitatively and qualitatively as compared to the bacteria associated with periodontal health. Numerous investigations have concluded bacteria associated with active periodontitis to be formulated primarily of motile gram negative anaerobic micro-organisms.[4,16,18,32,33,41,42,75,82-84,99] Listgarten et al.[41,42] and numerous other investigators[4,32,33,41,42,74,80,82,83,97] have supported this concept.

A study conducted by Listgarten and Leven[42] was performed to monitor and record the composition of subgingival microflora. Determination of whether microbial changes preceded or followed detectable deterioration of the clinical status of a group of chronic periodontitis susceptible subjects was studied. The prediction of susceptibility to future periodontal breakdown occurred through use of clinical or microbiological measurements.

Nineteen patients who had previously undergone surgical therapy, and who were receiving maintenance therapy of three to six months, participated in the study. The following baseline data were collected: Gingival Index scores, Plaque Index scores, probing depths, recession measurements, and subgingival microbial samples viewed under darkfield microscopy. All subjects received a prophylaxis and then returned for evaluation of baseline data at two-month intervals for one year. At the end of one year, another prophylaxis was rendered.

Statistical analysis of the data was performed for variations in mean measurements among the subjects and examinations. No significant differences occurred in the mean proportions of any bacterial group among the seven examinations rendered over a 12-month period. Significant differences existed in the mean bacterial populations among samples with spirochetes illustrating the largest F value at a 0.001 level of significance. Positive correlations were established between proportions of spirochetes and pocket depth measurements and between proportions of motile rods, and Plaque Index and Gingival Index scores. A correlation was present among the number of lost teeth per patient and the spirochetes, or spirochetes and motile rods, when the means of either the first, or all seven examinations were utilized. A positive correlation existed also between the lost teeth and probing depth measurements.

An increased probing depth possessed the likelihood of

paralleled further deterioration. However, the microbial proportions were found to provide increased quality of discrimination between disease-resistant and disease-susceptible subjects. The proportion of spirochetes with or without motile rods in samples obtained at baseline was found to be a good predictor of the periodontal deterioration of one or more teeth within the next year. This relationship may provide the clinician with an initial practical means of determinating the association of susceptibility among patients treated for adult chronic periodontitis and the existence of future periodontal breakdown.

A descriptive cross-sectional epidemiologic study of the subgingival microflora, performed by Keyes and Rams,[32] involved a total of 101 patients with oral conditions varying from excellent periodontal health to marginal gingivitis to advanced destructive periodontitis. Plaque samples were retrieved from the two sites of deepest pocket depth in each subject, placed on a slide, and examined for motile forms and crevicular polymorphonuclear neutrophil (PMN) leukocytes. The microbial complexes from the diseased sites varied in five ways when compared to those from healthy sites: (1) morphotypically, (2) quantitatively, (3) organizational patterns, (4) kinetically, and (5) pyogenic patterns. Keyes and Rams[32] confirmed that motile forms, particularly organized spirochetes, gliding rods, and amoeba were unique distinct features located in the plaque associated with destructive periodontitis. Unorganized motile forms, including clock arms, spiral rods,

and spinning rods were the predominant organisms associated with marginal gingivitis. Subjects with excellent periodontal health harbored low numbers of highly motile cocci and small motile rods. An absence of spirochetes, brush formations, larger motile rods, amoeba, or trichomonas were exhibited in plaque affiliated with periodontal health. These results lend support to the concept of microbial specificity throughout the development of periodontal lesions.

Research has shown that healthy gingival sulci harbor a unique and distinctive microflora.[22,32,34,40,41,83,99] The bacterial types associated with periodontal health predominantly include coccoid cells and gram positive organisms. Greenwall et al.[22] support this finding through darkfield microscopic evaluation of subgingival plaque samples. Several parameters were examined to determine the differences between clinical and microbiologic effectiveness of a nonsurgical treatment for periodontitis (the Keyes' method) and conventional oral hygiene. The nonsurgical treatments were compared in patients with periodontitis who were treated either by scaling and root planing alone or by scaling and root planing in combination with surgical pocket elimination. Examination through darkfield microscopy was performed within one hour of sampling. Sampling took place prior to scaling and at intervals of two, four, six, and eight weeks. Four categories of bacterial morphotypes were utilized: coccoid cells, others (straight rods, filaments, fusiforms), motile rods, and spirochetes. Results showed an

increase in the relative proportion of coccoid cells between zero and four weeks and a decrease from four to eight weeks. Changes for the coccoid cells between zero and eight weeks were not statistically significant. The category of others (straight rods, filaments, fusiforms) had a tendency to increase slightly from zero to eight weeks. This increase in straight rods, filaments, and fusiforms was statistically significant for the nonsurgical group only. A decrease in the proportion of motile rods was noted for all groups between zero and four weeks. No significant differences were present at zero to eight weeks for the proportion of motile rods. The proportions of spirochetes were significantly lower at zero, four, and eight weeks in the surgery group patients. The nonmotile flora, which consisted of cocci, straight rods, filaments, and fusiforms, was significantly higher for the surgery group at zero, four, and eight weeks. Overall, the proportion of coccoid cells increased following scaling and root planing, while there was a decrease in the relative proportions of spirochetes and motile rods. The resulting microbial proportions were significantly greater for patients treated surgically as compared to those treated with the Keyes' method of oral hygiene. These results indicated that the Keyes' method of oral hygiene was not significantly different in effectiveness than conventional oral hygiene procedures.

Direct phase-contrast microscopic examination studies by Keyes and Rams[32] have correlated the existence of loose networks of filaments and cocci, and absence of motile

bacterial forms and spirochetes, with excellent periodontal health. Keyes et al.[34] found supporting evidence through the conclusion of no existing relationship between excellent periodontal health and large numbers of spirochetes, larger motile rods, and white blood cells. Keyes et al.[34] reached these conclusions following examination of sulcular microbiota of patients utilizing a prescribed oral hygiene regimen. Microbial analysis included repetitive recording of the following bacterial types: white blood cells; motile cocci, small and large; motile rods, one to ten microns, and over ten microns; flexing rods, thin and thick; spiral rods; "arm-clocks"; spirochetes; brush configurations; trichonomas; amoeba; and others. These results may contribute to the theory of different microbial populations associated with periodontal health and disease. The possibility also exists that little benefit was achieved through the investigation by Keyes et al.[34] due to several factors which threatened the validity: no establishment of experimental and control groups; use of a limited sample population; a need for published data and statistical analysis; lack of documented intra- and interrater reliability; and void of an overall valid research design.

A study conducted by Listgarten[40] supports the previous assumption that microbiota vary in accordance with periodontal status. An electron microscope was used to observe samples of bacterial plaque obtained from gingival crevices of varying health states. In agreement with Keyes et al.,[34] Listgarten [40] found no flagellated cells or

spirochetes in samples from normal, undiseased crevices. Filamentous bacteria were more numerous in the gingivitis samples as compared to the undiseased samples. Flagellated bacteria and spirochetes were found in gingivitis. The periodontitis samples exhibited a transitional zone between the dense filamentous plaque and the largely motile subgingival plaque. These diseased samples also presented large numbers of spirochetes and "bristle brush" configurations. In accordance with Keyes and Rams,[32] who concluded five variational categories in which microbial complexes of diseased sites varied with those of healthy sites, Listgarten[40] has suggested several criteria: (1) certain microbial flora may be compatible with periodontal health; (2) varying degrees of periodontal disease possess associated different microflora; (3) the structure and composition of the supragingival flora possesses a definite difference from that of the subgingival flora; (4) microbial flora alterations also exhibit an increase in filamentous bacteria, mobile bacteria, spirochetes, and "bristle brush" configurations as periodontal disease increases in severity.

Significant differences between periodontally diseased and relatively healthy sites in their clinical and microbial features likewise have been demonstrated by Listgarten and Hellden.[41] The relative distribution of bacteria at clinically healthy and periodontally diseased sites was investigated utilizing 12 patients with advanced periodontal

disease. Subgingival microbial samples were obtained with a curette from each patient. Two relatively healthy and two periodontally diseased sites were included. Darkfield microscopy was used to classify microbiota as coccoid cells, straight rods, filaments, fusiforms, curved rods, spirochetes, and motile rods. The Gingival Index, Plaque Index, probing depth, and gingival fluid flow were recorded for each area sampled. Results obtained indicated the existence of significant differences in the microbial flora of clinically normal and diseased sites. A predominance of coccoid cells was present at healthy sites (74.3%), while spirochetes were rare in occurrence (1.1% or less). Diseased sites were dominated by motile rods (12.7%); curved rods (1.7%); and small (12.6%), medium (18.5%), and large (6.7%) spirochetes. A ratio of 1:49 of motile to nonmotile cells existed in the normal sites, while a 1:1 ratio was demonstrated in the diseased sites. Results have indicated a different microbial flora present for healthy sites as compared to the flora associated with diseased sites.

Slots[83] similarly obtained results that conclude a different microbial population for gingival health and diseased states. A total of seven to nine patients were examined in each of four clinical entities: healthy periodontium, gingivitis, advanced adult periodontitis, and juvenile periodontitis. Gram positive organisms dominated the scant microflora associated with the healthy gingival sulcus (85%). Through the development of gingivitis, an

increase was observed in the total number of gram negative organisms (45%). Microflora associated with advanced adult periodontitis consisted mainly of gram negative anaerobic rods (75%). These findings supported development of the following conclusions: (1) proportions of gram negative and anaerobic organisms in subgingival bacterial deposits increase with an increased severity of periodontal disease, and (2) different clinical entities of periodontal disease are associated with different subgingival bacterial deposit compositions.

Additional supporting evidence of the association of certain microbial types and various stages of periodontal health and disease has been demonstrated by Tanner et al.[99] Plaque samples were collected by an anaerobic gas-flushed syringe from a total of 21 sites in eight patients. The samples were anaerobically dispersed, diluted, plated, and incubated for 7 to 21 days in an atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. Colonies on plates containing 20 to 50 isolates were chosen, repeatedly restreaked, characterized, and identified. The differences between the bacterial populations in the groups were determined. Confirming results of previous studies, the subgingival microbiota in minimal-diseased sites was highly populated with gram positive organisms. The subgingival microbiota found in advanced destructive periodontal sites was predominated mainly by gram negative rods.

Armitage et al.[4] produced supporting results through

investigating the correlations between various clinical assessments of inflammatory periodontal disease and the percentage of motile bacteria in subgingival flora gathered from varying states of periodontal disease. While darkfield microscopy was utilized to determine the spirochete and motile bacterial percentages, the following were recorded for clinical assessments: Plaque Index, gingival exudate, Gingival Index, bleeding tendency, pocket depth, connective tissue attachment loss, and Periodontal Disease Index. Once again, clinically healthy sites exhibited a very small percentage of motile bacteria (below 1%). Positive correlations that were statistically significant ($p<0.001$) were found between the percentage of subgingival spirochetes and each of the following: Plaque Index ($r=0.54$), gingival exudate ($r=0.61$), Gingival Index ($r=0.57$), bleeding tendency ($r=0.59$), pocket depth ($r=0.56$), connective tissue attachment loss ($r=0.54$), and Periodontal Disease Index ($r=0.61$). The percentage of motile bacteria in the subgingival flora, while significantly related to periodontal disease severity, could be accounted for by variations in the percentage of spirochetes, thereby illustrating statistically significant correlations between subgingival spirochetes and the clinical measures assessed.

The composition of the subgingival microbiota after periodontal therapy has been investigated by Rosenburg et al.[74] Three quadrants exhibiting moderate periodontitis

(radiographic bone loss, and pockets of at least five millimeters around one or more teeth) were chosen in individuals with chronic periodontal disease. The Plaque Index, Gingival Index, pocket depth, and microbial data (coccoid cells, motile rods, spirochetes, and others) were recorded initially, and following scaling, root planing, and occlusal therapy. An inverse bevel surgical flap was performed, and data again recorded. The percentages of coccoid cells were increased significantly from one visit to the next, while percentages of motile rods and spirochetes decreased. These results represent sixteen of the eighteen patients treated. The two remaining patients illustrated a decrease in proportions of coccoid cells and an increase in proportions of spirochetes and motile rods. A positive association existed between spirochetes alone or with motile rods and probing depth. A negative association existed for the coccoid cells and pocket depth, Plaque Index, and Gingival Index scores. Patients were not treated or recalled at identical rates due to health complications and other reasons not listed. Despite the variation in the rate of delivery of care, clear-cut proportional microbial differences were illustrated through a decrease in coccoid cells and corresponding increase in motile rods and spirochetes. Microscopic proportions changed significantly from one examination to the next, while clinical measurements showed significant changes only between baseline values and values following surgical completion. Following therapy,

directional changes in Plaque Index and Gingival Index scores and pocket depth measurements bore an inverse relationship to proportions of spirochetes or motile rods. The sampling microbiological technique of pooling samples from six surfaces provided a representative estimate of the subgingival microbial composition in an individual at a given time. This technique illustrated a strong correlation with the entire dentition's clinical and periodontal status. Data collected in the investigation supports previous conclusions stating positive relationships among spirochetes, motile rods, and periodontal disease severity.

A predominance of coccoid cells and gram positive organisms in periodontally healthy gingival sulci has been shown to exist in the previously described studies.[4,22,32,34,40,42,74,83,99] The development of gingivitis was shown to be accompanied by an increase in the gram negative organisms, filamentous bacteria, flagellated bacteria, and spirochetes. Adult periodontitis continued this bacterial pattern by presenting a flora dominated by gram negative bacteria, filamentous bacteria, motile and curved rods, large numbers of spirochetes, and white blood cells. Destructive periodontitis was characterized by highly organized microbial patterns, as compared to the unorganized patterns associated with gingival health.[32] It may be concluded that the proportions of organized gram negative, anaerobic, and motile organisms present in subgingival bacterial deposits increase simultaneously with periodontal disease severity.

These organisms are not associated with periodontal health, of which coccoid cells and gram positive microorganisms are indicative.

A variation of microbial flora also has been indicated to occur between individual patients and individual sites.[12,13,32,36,41,48,58,63,73,74,83,84,99] Bacterial variability within diseased periodontal sites in patients with chronic periodontitis was examined by Evian et al.[13] Fourteen patients who possessed moderate to advanced untreated periodontal disease were studied. Along with probing depth, Plaque Index scores, and Gingival Index scores, microbial samples were measured by darkfield microscopy. Microbial samples were collected with a Gracey curette from the surface exhibiting the greatest depth in each sextant of the jaw. Four categories of microbial forms: coccoid cells, motile rods, spirochetes, and others were recorded. The calculation of correlation coefficients between the proportions of microbial types and clinical parameters was performed to determine the presence of correlations significant at the .05 level. Results illustrated considerable variation for each microbial type when comparing the proportions of different bacteria among the six sites in any individual dentition. Significant differences were noted for all bacterial groups among individual patients. When the percentages of spirochetes or motile rods were compared between sites, no significant differences were found; however, if grouped together, a

difference of borderline significance was demonstrated. Significant differences were found between sites for the coccoid cells. When mean percentages of spirochetes were examined, a direct correlation was found with increased pocket depth, Plaque Index scores, and Gingival Index scores. Mean percentages of coccoid cells also possessed a tendency to follow a reverse pattern. When measurements from individual sites were analyzed, no significant correlations could be demonstrated. The variance in data was due mainly to differences between individual subjects, rather than sites. Evian et al[13] postulated that the large variance present among subjects and sites was partially due to the cyclical nature of periodontal disease and to the lack of synchrony between remissions and exacerbations present at different sites in individual patients. An indication exists for further research utilizing a larger population sample.

The study performed by Listgarten and Héllden[41] concerning the relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans produced evidence to support the differential bacterial concept. The results obtained in the study were clearly indicative of the association of a different flora with healthy and periodontally diseased sites in the same patient population. Slots[84] investigated the predominant cultivable microorganisms of pockets associated with advanced periodontitis of eight patients. A total of 475 isolates were examined,

and their mutual proportions differed considerably among the samples. These results parallel those of other studies that have involved the microscopic evaluation of several bacterial samples gathered from several independent sites within gingival sulci of each subject.[4,13,32,42,58,84,99] Through examination of these results, one may identify the presence of definite quantitative and qualitative microbial differences among the individual sites of each subject and among individual subjects. Studies that have involved the comparison of bacterial samples among several individuals present additional supportive evidence.[4,13,32,34,40,42,58,74,83,84,99]

Variations of periodontal disease further occur among the exhibitions of the disease process itself.[4,13,20,32,83] A total of 22 patients with existing periodontal pockets greater than four millimeters participated in a study conducted by Goodson et al.[20] The attachment level at two sites on each tooth in all patients (a total of 1,155 sites) was measured every month for one year. A regression analysis was applied to data obtained from each site to determine the existence of statistically significant trends in attachment level change. A large percentage (82.8%) of the periodontal pockets did not exhibit a significant change in attachment level when monitored for 13.2 months. Significantly deeper measurements were found in 5.7% of the sites, while 11.5% illustrated significantly shallower measurements ($p<0.01$). Approximately 50% of the sites that

illustrated increased pocket depth displayed a cyclic deepening followed by spontaneous recovery to their original depths. Fifteen subjects illustrated sites that became significantly deeper while other sites became simultaneously significantly shallower. An arrested form of periodontal disease, shown by six subjects, did not present deepening sites; however, 11 to 36% of the sites became significantly shallower. Results suggested that a possible dynamic characteristic of periodontal disease, exacerbation, remission, and inactivity periods, may exist. These results support the dynamic bacterial populations and associated various clinical entities, [4,32] disease activity,[83] and cyclical nature of the disease.[32]

Many methodologies currently exist that may be utilized in the determination of periodontal disease. Traditional methods such as periodontal pocket measurements, radiographic determination, and clinical index recordings are static evaluations with limited usage in the determination of disease activity.[23,29,33,34] Gingival crevicular fluid contents may be utilized as a method for determining tissue changes in the periodontium, but lacks definite proof of effectiveness.[23] Tissue changes themselves are important in understanding the disease process, yet are of limited indicative value of the actual determination of disease activity.[23] Changes that occur in the gingival tissues, such as multiple inflammatory foci development and inflammatory cellular response changes have served as means for

advancement of man's knowledge concerning periodontal disease. The immunoglobulin content of the tissues do not present parallel changes in accordance with disease activity. These traditional clinical measurements of probing, radiographs, clinical indices, crevicular fluid contents, and tissue changes have been used in various combinations in many investigations for the purpose of determining various aspects of periodontal disease. These aspects may include presence, severity, and future monitoring of the disease.[4,13,22,23,33,34,41,42,44,58,82] The determination of disease activity through changes or substance identification in the systemic circulation may be a valuable process for the clinician.[23] A measurement of the present oral periodontal status may be assessed in this manner, as compared to already past destruction measurements. Obtained information from the microbial sampling serves as a means of determining the existence of a therapeutic necessity, or if past treatment has been effective. This determination allows possible evaluation of periodontal status.[41,42,82]

The Microbial Species Bacteroides melaninogenicus, Its Relationship with Periodontal Disease, and Its Detection Through Plated Culture Media

Many investigations performed, which involved selective isolation of anaerobic microbial species, have utilized plated culture broth as a growth medium.[35,51,52,57,59,60,71,81,90,95,98,109,113,114] The results obtained provided

data that was cumulative and macroscopic in nature.[102] Bacteroides, an anaerobic gram negative microbial species associated with periodontal disease, frequently has been evaluated through utilization of culture media.[8,17,35,51,52,55,57,59,71,81,83,85,86,90,99,109,113,114]

Socransky and Gibbons[90] investigated the role of Bacteroides melaninogenicus in synergistic anaerobic infections. Cultures both pure and mixed in nature were innoculated into guinea pigs (200-250 g) to determine infective ability. Development of localized transmissible abscesses, spreading necrotic lesions, or death represented positive infectivity. Noninfectivity was evidenced through hard, nodular, nontransmissible caseous abscesses or mild inflammation. Microbial samples were obtained from the gingival crevice and human fecal flora. Samples from the gingival crevice were obtained at the apical portion of a pocket with a sterile curette, dispersed by sonic oscillation, diluted, and plated on heart infusion blood agar. The Bacteroides melaninogenicus present in the fecal flora were prepared in a different manner. One gram samples of freshly collected human feces were homogenized in heart infusion broth in a glass tissue grinder and tested directly for infectivity. A portion of each suspension was serially diluted in broth and placed on heart infusion blood agar plates and heart infusion agar without blood.

Elimination of Bacteroides melaninogenicus from naturally occurring mixtures of human indigenous bacteria was

performed through three experimental methods. The first approach involved isolation in pure culture of bacteria present in high numbers following deletion of Bacteroides melaninogenicus, which allowed complete sample representation in the final inoculum. The second method was dependent upon the hemin nutritional requirement of Bacteroides melaninogenicus. Deletion occurred through serial passage on media free of hemin. This second method allowed organisms to be present in low concentrations throughout transfer and into the final inoculum. A third method for eliminating the microbial species from complex mixtures of human indigenous bacteria involved the use of germ-free animals, which possessed advantages similar to media selection.

Results of all three methods were similar: noneffectiveness in human indigenous bacteria mixtures lacking Bacteroides melaninogenicus. Infections observed were dependent upon the addition of pure cultures or naturally occurring strains of Bacteroides melaninogenicus. Data obtained in this investigation suggested that infections which before were considered bacteriologically nonspecific in nature may depend upon the presence of Bacteroides melaninogenicus. These results relate to the investigation to be performed through illustration of the correlation among Bacteroides melaninogenicus and infection, especially concerning samples obtained from the gingival crevice.

The subgingival microflora associated with periodontal

disease and its inhabitant of Bacteroides species has been investigated through past research.[51,52,57,81,86,98,109,114] The bacteriology of moderate chronic periodontitis was studied by Moore et al.[57] Twenty-two subjects possessing a total of 38 affected sites of moderate periodontitis were included. Each of the sixty samples taken (using Morse 00 scalers) were transferred immediately to prereduced, anaerobically sterilized broth under oxygen-free carbon dioxide. Samples were dispersed, diluted, and cultured. Types of cultures included role tubes of 0-4 medium and petri plates of 0-4 medium containing rabbit blood. Following an incubation time of five days, bacterial colonies were selected in a randomized pattern to provide a representative cross section of flora. A total of fifteen colonies were selected from each culture. Electrophoretic analysis of soluble cellular proteins, chromatographic and biochemical procedures, and tests for facultative or nutritionally demanding taxa were utilized for identification. Statistical analysis was performed through Good's analysis for coverage and Good's L - test.

A total of 171 taxa in 29 bacterial genera were identified as distribution of the isolates. Sixty-two of the described species accounted for 65% of the subgingival microflora. Thirty-seven of the 136 taxa detected in the subgingival flora of moderate periodontitis were in equal or greater concentrations in subgingival samples from healthy gingiva. These 37 taxa, therefore, may have been considered

unimportant as direct causative agents of periodontal disease. A total of 85 subgingival taxa were more frequently in the subgingival flora of moderate periodontitis, as compared to healthy subgingival flora or moderate periodontitis supragingival flora. Fifty-seven of these species occurred at concentrations of less than 0.5 percent and remain in the consideration of an etiologic source. Included in the subgingival moderate periodontitis flora were Fusobacterium sp., Peptostrepto coccus sp., Eubacterium sp., Bacteroides sp., Wolinella sp., Lactobacillus sp., Actinomyces sp., Selenomonas sp., Haemophilus sp. Bacteroides melaninogenicus was present in a concentration of 0.2 percentage. All of the taxa listed were not believed to be clinically significant in the etiology of periodontal disease. Results indicated that the ratios of several predominant species differ in moderate and severe periodontitis, which possess similar etiologies.

The isolation, characterization, and identification of black-pigmented Bacteroides endodontalis was investigated by Van Winkelhoff et al.[114] Strains were isolated from various origins including dental root canals, oral submucous abcesses, periodontal pockets, clinical specimens, infected hemorrhoids, feces, leg wounds, and dental plaque. A nitrogen-flushed syringe was the instrument of connection for samples from dental root canals. Following collection procedures anaerobically sterilized 0.25 strength Ringers solution was used to transport the root canal samples.

Sterile cotton-wool swabs were used for collecting samples from submucous periapical abcesses, which were then placed in charcoal transport medium. Colonies plated on horse blood agar were examined following 5, 7, and 14 days of incubation.

Fermentation of carbohydrates, catalase production, hemagglutination, growth in air, and several biochemical properties were examined. Deoxyribonucleic acid (DNA) composition and homology, enzyme patterns, and growth in various gas mixtures were also investigated. Antiserum production occurred for whole cells of one B. endodontalis strains.

Results showed a total of twelve B. endodontalis strains isolated from dental root canals or submucous periapical abcesses. Cultures obtained consisted of obligate and facultative anacrobic streptococci, Fusobacterium species, and black-pigmented Bacteroides species. The presence of B. endodontalis in endodontic infections and submucosal abcesses was a constant source vital to knowledge resulting from this investigation.

The virulence of black-pigmented Bacteroides strains from periodontal pockets and other sites in experimentally induced skin lesions in mice was investigated by van Steenbergen et al.[109] Microorganisms were obtained from isolated sites consisting of periodontitis, dental root canal, dental plaque, laryngotomy wound, sputum, great toe, oral specimen, cervical specimen, faeces, empyema, and

legwound. The strains were characterized as B. gingivalis, B. assaccharolyticus, and B. melaninogenicus. Maintenance of strains occurred by weekly subculture on 5% horse blood agar plates supplemented with 5 ug/ml hemin and 2 ug/ml medium. Growth of cultures was performed in liquid BM medium. (The composition of BM medium was not described). Cultures were harvested by centrifugation and resuspended in sterile BM medium. The average number of bacteria in the suspension was $5x10^{10}$ viable cells per milliliter. Atmospheric conditions in an anaerobic chamber were utilized for all harvesting and preparation of suspensions. A volume of 0.1 milliliters was injected subcutaneously into the backs of two mice. The mice were sacrificed after four days and historically examined utilizing dorsal and ventral skin fragments.

Results revealed a necessity for concentrated bacterial suspensions for a lesion to be produced. B. gingivalis strains were characterized by inducing a spreading inflammation and a phlegmonous abscess or a gravity abscess. An abscess at the site of the injection was rarely seen with B. gingivalis. Oral strains of B. melaninogenicus subsp. intermedius, however, always produced a localized abscess at the site of injection. B. melaninogenicus subsp. melaninogenicus were less virulent and produced few inflammatory cells. B. asaccharolyticus (nonoral isolates) produced a localized abscess at the injection site. Asaccharolytic black-pigmented Bacteroides strains isolated

from necrotic dental pulp produced a thin layer of inflammatory cells, spreading over the entire skin fragment. Observations presented suggest important roles in periodontal disease for all three species of Bacteroides.

The ability of cells of Bacteroides melaninogenicus subsp. asaccharolyticus to adhere to surfaces that might serve an important function in the initial colonization of the mouth and subsequently, in periodontal pockets, was investigated by Slots and Gibbons.[86] Several strains of microbiota were isolated: B. melaninogenicus subsp. asaccharolyticus, B. melaninogenicus subsp. intermedius, B. melaninogenicus subsp. melaninogenicus, Fusobacterium nucleatum, Capnocytophaga, Eikenella corrodens (rough colony and smooth colony), "corroding" Bacteroides strains 401 and 402, Campylobacter strain 288, Vibrio strain 371, A. israelii, Clostridium sporagenes, Actinomyces naeslundii C2, A. viscosus, S. sanguis, S. salivarius, S. mitis, and S. mutans. All organisms were maintained by weekly transfer on Todd-Hewitt agar (BBL) supplemented with sheep blood, hemin, and menadione.

Sixty-eight strains of B. melaninogenicus were examined for hemagglutinating activity. Forty-seven of the strains revealed strong agglutinating activity of human type A erythrocytes and failed to ferment glucose; thereby representing the subspecies B. asaccharolyticus. Twenty of the 21 nonhemagglutinating strains were fermentative. Following examination of representative asaccharolytic strains for the

presence of pili, the researchers found that the presence of surface pili did not directly correlate hemagglutinating activities.

B. melaninogenicus strains representative of all subspecies were found to attach to human crevicular epithelial cells. Pooled human serum almost completely inhibited attachment, thereby suggesting inhibition of attachment by serous components in crevicular fluid. Experimentation in vitro revealed data that showed B. melaninogenicus cells in saliva or serum containing oral environments as possibly possessing a feeble capacity of attachment to tooth surfaces containing adsorbed salivary components. B. melaninogenicus strains including subspecies asaccharolyticus, intermedius, and melaninogenicus attached well to surfaces of gram positive bacteria without inhibitory interferences by saliva or serum. The suggestion was made that surfaces of gram positive bacteria may be important receptors for attachment of B. melaninogenicus strains and other gram-negative bacteria in the oral environment. Studies both in vivo and in vitro in nature revealed attachment to receptors on surfaces of other dental plaque bacteria by B. melaninogenicus subsp. asaccharolyticus.

Theorization of the colonization of bacteria in a periodontal pocket was made by Slots and Gibbons.[86] Upon initial introduction into the mouth, some cells of the species must begin colonization through attachment to an oral surface exposed to saliva. The present or eventual

occurrence of this attachment on a tooth surface would permit the spreading proliferation of dental plaque to provide entrance into the periodontal pocket. Locomotion (motile organisms), masticatory forces, oral hygiene procedures, or dental instrument utilization may provide means of transfer. The colonization of bacteria in dental plaque supports the necessity of supragingival plaque removal for the reduction of gram-negative bacteria colonization.

The influence of supragingival plaque control upon subgingival microflora composition was supported by Siegrist and Kornman.[81] Ligature-induced periodontitis in four adult female Cynomolgus monkeys with intact dentitions and completely formed and erupted third molars was studied. Baseline examinations using the Plaque Index and Gingival Index, radiographs, and photographs revealed generalized gingivitis and minimal evidence of periodontitis. Orthodontic elastics were placed around the four first or second bicuspids of each animal to induce periodontal disease and were left in place for 6-8 weeks to achieve 3-5 mm pocket depths. Following removal of the elastics, supra- and subgingival microflora were allowed to stabilize for four to nine weeks. Samples were then taken. Supra-gingival plaque removal was accomplished on experimental teeth three times a week for six weeks using a rubber cup, pumice, and dental floss. Opposite sites served as control sites and received no treatment. Clinical and microbiological data were obtained at weeks zero, two, and six. In the first and

second animals, oral hygiene procedures were re-initiated after four and five weeks, respectively, on the former control sites; thus, the former test sites then served as controls. A total of twelve control and twelve test sites were obtained. The monkeys were anesthetized with ketamine/atropine anesthesia to provide satisfactory working times of 30 to 40 minutes. Three sterile fine paper points inserted to the sulcus base for ten seconds was the method of subgingival plaque collection. Upon removal, apical portions of the paper points were placed in one milliliter of reduced transport fluid without ethylene-diamine-tetraacetate, vortexed for 60 seconds, and taken into an anaerobic glove box. A smear was then created. Samples were then dispersed, diluted, and plated on nonselective, reduced enriched trypticase soy agar, and inoculated in an anaerobic chamber for five days. Twenty to 50 colonies were characterized by color, shape, circumference, and frequency. One colony was chosen and subcultured.

The change in total cultivable flora was calculated for the various microbiological groups. The pairwise t-test for correlated data was used to evaluate the difference between clean and non-cleaned sites. Gram-positive cocci and rods decreased, whereas gram-negative cocci and rods increased in cleaned as well as non-cleaned sites. No statistical difference for these organisms was shown between cleaned and non-cleaned sites. Black-pigmented Bacteroides decreased in cleaned sites at weeks two and six and showed

a statistically significant difference between cleaned and non-cleaned sites at week two. Fusobacteria species increased in cleaned sites at weeks two and six and decreased in non-cleaned sites at week two. A significant decrease was shown in the total cultivable flora. No clinical differences or changes were seen in the Gingival Index and pocket depth, despite a reduction in the Plaque Index. Results indicate supra-gingival plaque control in the presence of periodontal disease may alter the sub-gingival microflora associated with periodontitis. This alteration may not alter clinical signs in the time period evaluated.

The microbial species of Bacteroides has several characteristics which distinguish its presence.[35,59,113] The production of a black hematin pigment when growth occurs in a blood-containing medium, a specific ultrastructural characterization, and a bacteriocin-like substance are some of the descriptors of Bacteroides species.

Woo et al.[113] examined the ultrastructure of three Bacteroides species to determine their characterization. Specific attention was given to the cellular surface and its possible role in cell-host interaction and pathogenesis. B. asaccharolyticus strains 536B, 2013, 2015, 2017, 2019, and 2021; B. melaninogenicus subspecies intermedius strain 2210; B. melaninogenicus subspecies melaninogenicus strain ATCC 15930; and B. fragilis strain ATCC 25285 were included in the study. Cultures were grown on solid medium

consisting of Todd-Hewitt broth plus 1.5% agar enriched with sodium succinate, hemin, menadione, and sheep blood and in liquid medium composed of Todd-Hewitt broth. Plate-grown cells were incubated for three to four days at 37°C in Brewer jars in an atmosphere of 90 percent hydrogen and 10 percent carbon dioxide. Liquid cultures were created by inoculation of growth from three-day culture plates and were incubated overnight. Bacteroides strains were transferred at weekly intervals to maintain culture viability. Microscopic evaluation was performed through oil immersion, phase-contrast optics with a Zeiss GFL microscope, and through electron microscopy.

Phase contrast microscopy revealed short rods with characteristic coccobacilli morphology to be representative of the Bacteroides strains. All strains revealed a 0.2 um "halo" quality of a capular structure and loose or torn extracellular material through phase-contrast microscopic evaluation.

Electron microscopy showed typical gram-negative morphology to be present. An outer membrane composed of a membrane bilayer of various widths was present for the different Bacteroides species examined. The region between the outer and inner surfaces of the cell membrane, the periplasmic space, was very thick (approximately 22-26 nm). Peptidoglycan was found beneath the inner surface of the outer membrane and within the periplasmic space. In all strains, the cytoplasmic region was composed of electron-

dense particles (presumed to be ribosomes) that were interspersed with the fibrous nucleoid. A multitude of thin fibers with extensive branching and fiber interconnection were found to radiate from all dimensions of the cellular surface. The electron-dense material found external to the surface of the outer membrane may possess biological or chemical capabilities in association with periodontal disease. More research is indicated in this area.

The detection, purification, and partial characterization of melaninocin produced by strains of Bacteroides melaninogenicus was described in a study by Nakamura et al.[59] A total of 23 B. melaninogenicus strains were isolated from gingival crevice deposits and identified as the following subspecies: melaninogenicus (10), asaccharolyticus (10), and intermedius (3). Examination of these strains then occurred for the production of growth inhibitory substances. Liquid culture medium consisting of Trypticase peptone, yeast extract, glucose, sodium chloride, potassium phosphate, thioglycolate, hemin, and menadione was used. Experimentation was carried out at 37°C for five days anaerobically in a glove box.

Examination of growth inhibition revealed that 13 out of 23 strains produced inhibitor effective against a minimum of one spectrum of strains. No significant activity in the culture supernatants of cellular localization of melaninocin within or on cells was determined. No inhibitory activity was detected in extracts from intact

cells. Only ultrasonic treatment liberated melaninocin in sufficient amounts. Following purification, melaninocin could be fully destroyed by heating at 65°C for ten minutes. Storage at -20°C for several months revealed no significant activity reduction. Melaninocin was shown to be effective in inhibiting several strains of oral indigenous bacteria: A. viscosus, A. naeslundii, S. mitis, S. salivarius, B. oralis, B. ochraceus, and non-melaninocin-producing strains of B. melaninogenicus. Bacteria found to be resistant to melaninocin were S. mutans, F. nucleatum, V. alcalescens, B. matruchotii, P. acnes, and C. parvum. Melaninocin, therefore, possesses inhibitory characteristics toward various microbial species of the oral cavity.

The physiological and ultrastructural characterization of Bacteroides capillus also has been investigated by Kornman and Holt.[35] Isolation of samples from severe localized periodontitis from three individuals was performed with curette and sterile fine paper points. The samples were placed in a reduced transport fluid and immediately placed into an anaerobic glove box. Samples were then sonified, diluted, and plated on enriched trypticase soy agar. Light microscopy and electron microscopy were utilized for observation.

The single most predominant isolate quantitated was a gram-negative anaerobic rod (40.6% to 72.4% of the microbiota). The isolates were given strain designations of 925.08, 938.11, 1288.01, and 1363.08 and were classified

as Bacteroides capillus. The strains were grown on blood agar in an anaerobic chamber in Brewer jar conditions. Colonies produced were clear, entire, circular, convex, approximately two to three millimeters in diameter, and light buff in color. Presence of a capsular exopolymer was shown when colonies appeared liquified following prolonged growth of five to six days. Phase contrast microscopy revealed short rods approximately 1.2 to 1.5 um in length and 0.58 um in width. India ink staining disclosed an extensive capsule surrounding all the cells. No motility was observed. Electron microscopy revealed a typical gram-negative morphology. The cell envelope of a wrinkled outer membrane was approximately 6.7 nm thick. An electron-opaque material filled the periplasmic space, and a thin peptidoglycon layer closely opposed to the cytoplasmic membrane outer surface. An additional layer to the gram-negative envelope was present and gave the appearance of one-half a membrane bilayer connected to the outer surface by "linkers" approximately 2-4 nm wide. A disorganized electron-opaque fibrous material filled the background of the cells. The additional external layer stained ruthenium red positive and consisted of an extremely fragile double layer approximately 7-8 nm thick. Long, ruthenium red hairlike fibers emerged from the surface of the outermost layer. An extensive thick, amorphous layer was shown to exist through scanning electron microscopy. All B. capillus strains, except 925.08, fermented a wide variety of

carbohydrates. When grown in the presence of glucose, all strains produced acetic and succinic acids. Tryptic and yeast extract digests proteins were required for growth of the obligate anaerobes. Addition of hemin to the growth medium resulted in a stimulation, but was not required. Maximum growth appeared to occur at 30-35^{o}C, while growth was inhibited at 45^{o}C. Hydrogen peroxide was neither decomposed nor formed by any of the strains examined. All B. capillus strains except 925.08 grew in the presence of NaCl and KCN and were inhibited by sodium fluoride. All strains showed sensitivity toward penicillin, ampicillin, and erythromycin and resistance toward kanamycin, refamin, tetracycline, streptomycin, and polymyxin B. The characteristics of this specific Bacteroides strain allow designation of its presence in various conditions found in the oral cavity. The distinctive hair-like fibers would permit one to establish its presence during the periodontal disease process.

The Bacteroides species possessed properties that carried destructive potential for several substances.[8,17,71,92,95] The characteristic of collagenolytic activity associated with Bacteroides melaninogenicus has been shown through investigations.[17,71] Gibbons and MacDonald[17] isolated thirteen oral strains of B. melaninogenicus from gingival scrapings and fourteen strains from anal swabs. Three rumen strains were provided by an outside source. All strains required hemin and/or menadione for growth and were

maintained by weekly transfers. Cultures were screened for collagenolytic activity, and hydrolysis of native collagen was shown through use of fresh or lyophilized cells from a broth culture. Determination of quantitative collagenolytic activity, a relatively pure homogeneous collagen substrate, was required. All thirty strains of B. melaninogenicus grown in trypticase yeast medium for fourteen days hydrolyzed reconstituted collagen gels. Strains varied in activity; however, oral strains were most active, for they digested an average of 55 percent of the collagen. Cultures of B. melaninogenicus attained maximal growth within 48 to 72 hours, but degradation of collagen gel did not occur until culture autolysis began and increased as autolysis continued. This evidence suggested an intracellular nature of the enzyme until release by autolysis. B. melaninogenicus was found to hydrolyze native collagen, azocoli, casein, egg albumin, and plasma protein. The optimal pH for collagenolytic activity was over 7.3. Greater acidic or alkaline conditions sharply decrease activity. Also, heat-liability exists for the enzyme. This optimal pH range for collagen hydrolization perhaps aids in the explanation of the chronic nature of periodontal disease.

Robertson et al.[71] assessed a variety of microorganisms of collagenolytic activity with particular emphasis placed upon members of the indigenous oral flora species of Bacteroides. Organisms were grown anaerobically at 37°C in

complete basal anaerobic broth. A second culture series was performed in which medium peptides were depleted. Light microscopic examination confirmed cell morphology and homogenicity. Organisms were characterized concerning gram-strain and morphology, acid production, nitrate and nitrite reduction, catalase activity, indole and ammonia production, urease and oxidase activity, motility, atmospheric growth, and acid end-products. Collagenolytic activity was assessed using ^{14}C-acetylated collagen broth. Results showed collagenolytic activity in all species of Bacteroides and Actinobacillus actinomycetemcomitans tested. A phenomenon that reflects autolysis also was observed when Bacteroides species reached the stationary growth phase and collagenolytic activity occurred in the media. This collagenolytic activity is a major feature of inflammatory periodontal disease progression.

Phagocytosis of five strains of Bacteroides melaninogenicus and two strains of Bacteroides gingivalis by human neutrophils was studied in vitro by Sundquist et al.[92] Microorganisms were isolated from root canals of teeth with necrotic pulp tissue and purulent periapical inflammation and were stored under anaerobic conditions. Growth occurred in PY-glucose broth for 48 hours. Chemiluminescence response was measured for all bacteria. Electron microscopy of polymorphonuclear leukocytes was used to determine if the chemiluminescence activity observed was associated with ingestion of the organisms exposed to B. melaninogenicus or

B. gingivalis. Phagocytosis by the polymorphonuclear leukocytes of four of the five B. melaninogenicus strains occurred within ten minutes. The B. melaninogenicus strain that was not ingested after ten minutes produced the lowest chemiluminescence response and illustrated a loosely bound network of intermediate electron density surrounding its cell wall. The B. gingivalis strain that produced the lowest chemiluminescent response had an electron dense layer covering its surface. An increase in the incubation time from ten to twenty minutes revealed observation of two to ten bacteria of the B. melaninogenicus strain in leukocyte sections, but only an occasional bacteria of the B. gingivalis strain. Phagocytosis is an important event in the host defense against invading bacterial pathogens. The possible resistance to phagocytosis of B. melaninogenicus and B. gingivalis could be the causative factor of the infectious properties.

Polyclonal B-cell activators of the subgingival flora could result in several B-cell responses: proliferation, polyclonal antibody production, and the release of osteolytic lymphokines (which includes osteoclast-activating factor).[8] Increased inflammation, bone resorption activation, and loss of periodontal support could then occur. Bick et al.[8] investigated the polyclonal B-cell activation induced by extracts of gram-negative bacteria isolated from periodontally diseased sites. Strains selected for testing included Fusobacterium nucleatum, B. melaninogenicus subsp.

melaninogenicus, B. melaninogenicus subsp. intermedius, Bacteroides gingivalis, Selenomas sputigena, Capnocytophaga ochracea, and Actinobacillus actinomycetemcomitans. In the author's samples to date, F. nucleatum was the most common species of the periodontally diseased subgingival flora, while B. melaninogenicus subsp. intermedius was the second most frequent organism. Bacterial strains were harvested from broth cultures in peptone-yeast extract broth with appropriate additives necessary for growth. Strains were then washed, suspended in a buffered saline-glycerol solution, and stored frozen until sonicated for use. Normal human peripheral blood lymphocytes (PBL) were obtained from individuals without periodontitis (as defined by loss of attachment). Each PBL culture was assayed individually and viability determined for each group (from pooled samples). Extracts of the isolated organisms were added to the cultures of PBL's for determination of polyclonal activating ability of the periodontally associated bacteria. Various doses of the bacterial extracts were added and cultured in a period of six days. Following six days, cultures were harvested and assayed for polyclonal antibody synthesis. All of the bacterial isolates demonstrated the ability to polyclonally stimulate PBL's to produce antibody. However, intensity of the lymphocyte response to a given extract did vary among cells from different individuals. Conclusions were reached that resident gram negative subgingival flora associated with periodontal

lesions possess polyclonal B-cell activators which may contribute to disease pathogenesis through the induction of B-lymphocytes to produce antibody, osteolytic factors, and perhaps other mediators of inflammation.

The capacity of lipopolysaccharides isolated from oral strains of Bacteroides, Fusobacterium, and Veillonella to stimulate resorption of fetal rat bone in culture was investigated by Sveen and Skaug.[95] Bacterial strains utilized for the isolation of lipopolysaccharides (LPS) included: Bacteroides melaninogenicus subsp. intermedius strain B10, Veillonella alcalescens strain Ve5, Veillonella parvula strain Ve9, Fusobacterium nucleatum strains F1 and Fev 1, and Bacteroides fragilis subsp. fragilis strain NCTC 9343. LPS was extracted and purified. Lipid A and polysaccharide preparations were derived from LPS. The final preparations of LPS, lipid A, and polysaccharide preparation were used to induce bone resorption. The basic culture medium consisted of Bigger's modified medium. Female rats were injected subcutaneously with 500 uCi of Calcium -45, Batch 27DB on the eighteenth day of pregnancy and sacrificed following 24 hours. The rats' fetal ulnae and radii were explanted as pairs for tissue cultures. Fetal bones were then washed in the basic culture medium, precultured, then cultured. One pair of bones served as the experimental pair while the other served as the control.

Results obtained showed variations in the capacity of the different LPS preparations to induce the release of ^{45}Ca

(radioactive isotope of calcium) from the prelabeled bones. Lipid A and the polysaccharide part of LPS-Fev 1 induced the release of ^{45}Ca and hydroxyproline. Comparison and weight basis showed the polysaccharide preparation to be more active than lipid A and the intact LPS, whereas lipid A showed the lowest activity. After two days in a culture medium containing endotoxin, histological preparations showed a sparsity of bone and an increased number of multinuclear cells in bone lacunae in comparison to bone found in the control medium. An increased loss of incorporated ^{45}Ca from fetal bones was present with the various LPS preparation and under strictly defined conditions. A dose-response curve was observed which indicated an inhibitory effect of LPS upon the calcium release when used in supraoptimal concentrations. The increased release of hydroxyproline was paralleled with increased release of ^{45}Ca. Conclusions may be drawn that LPS preparations were potent bone resorption stimulators due to the release of ^{45}Ca and loss of collagen. The strong indications of participation of metabolic processes in living cells, the fact that the metabolite lactate is a nonspecific indicator of metabolic activity of culture cells, and the histologic findings of polynuclear cells (identified as osteoclasts), together with the bone matrix scarcity, indicated an active bone resorption.

Many investigators have contributed to the association of Bacteroides with the presence of periodontal disease.[8,

17,35,51,52,55,57,59,71,81,83, 85, 86, 90, 99, 109, 113, 114

Several factors are present which support this association: surface adherence,[86] hemagglutinating activity, hematin production, [60] ultrastructural characterization, [35,113] melaninocin production, [59] degradation of collagenous sulphates,[13,71] and osteolytic activity.[8,95] These investigations have added greatly to current knowledge of periodontal disease; however, much research remains to be performed concerning the exact roles bacteria play in the development of periodontitis and in the ultimate destruction of the periodontium.

Löe's Gingival Index and Plaque Index Systems for Assessment of the Gingival Condition

The Gingival Index Systems was created by Löe[45] for the purpose of assessment of the gingival condition. The index was designed for clear distinction between the quality of the gingiva and the location as related to the four areas (buccal, mesial, distal, lingual) which comprise the circumference of the gingival margin. Only qualitative changes are measured by the Gingival Index as opposed to periodontal pocket depths, osseous decrease, or other changes occurring in the periodontium.

The Plaque Index was developed on the principle of distinguishing clearly between the severity and location of soft debris aggregates. A second reason for the development of the Plaque Index was to create a system to identically match the Gingival Index.

Both indices may be utilized to assess respective prevalence and severity measurements in large population groups as efficiently as individual patients. Additionally, each index may produce the score for a given area of a tooth, a single tooth, groups of teeth, and/or the individual. The correspondence present between the Gingival Index and Plaque Index, the sensitivity represented by each, and the reproducibility may greatly contribute to many preventive and therapeutic measures evaluations.

Many investigations have relied upon the Gingival Index and/or Plaque Index to provide them with statistically valid accurate data.[4,13,37,38,42,47,74,87,89,101,112] Investigation of experimental gingivitis in man by Löe et al.[47] and Theilade et al.[101] centered upon bacterial plaque deposits and gingival inflammation, thereby relying upon both indices. Lindhe et al.[37,38] utilized the Gingival Index to determine the effect of supervised oral hygiene on the gingiva of children.

Evaluation of a training program for the Gingival Index and Papilliary-Marginal-Attached (PMA) index was investigated by Alexander et al.[1] Three examiners who previously had achieved inter-examiner agreement were separated for a four-month period. The four-month separation permitted neither discussion nor joint examinations concerning either index. Following this time period, scoring of the labial gingiva of 16 subjects with both indices occurred. Examiners again standardized their scoring through an

additional patient examination. Each trained instructor then taught a selected pupil in the use of each index. The original training program consisted of the following: (1) preliminary reading of relevant scientific articles, (2) two hours of lecture and discussion, (3) three hours of clinical instruction, (4) four three-hour concurrent practical clinic sessions without the presence of the instructor, and (5) three additional hours of clinical instruction. Gingival inflammation on labial regions of various groups of 21 subjects were analyzed by each pair of examiners. Inter-examiner agreement achieved by each pair was assessed and modifications made to the training program.

Mean Gingival and PMA Indices were calculated from individual tooth scores of each examiner. Following the four month period with no contact, examiners one and two no longer were comparable on the Gingival Index ($p<0.001$), while the PMA index remained in agreement for both indices ($p<0.1$). Examiners one and three remained in agreement for both indices ($p>0.1$); while examiners two and three failed to maintain agreement either in the Gingival Index ($0.01>p>0.002$) or the PMA index ($0.02>p>0.01$). The training program permitted the first examiner and student to agree on each index measurement. Examiners two and three and their respective students agreed only on one index each: PMA index, examiner two and his student; Gingival Index, examiner three and his student. Additional exercises were given to these two examiners and their students which

permitted reliability only to be achieved by pair number three. Alexander et al[1]. concluded that time and care must accompany selection of an examining team for the production of inter-examiner agreement. No indication of an easier comparability achievement for either the Gingival Index or the PMA index was found. This demonstrates that the potential for interrater reliability poses a very good chance of achievement with both index systems.

Widespread use of Löe's Gingival Index and Plaque Index aid in the establishment of its utilization as a valid and reliable instrument of measurement.[4,13,37,38,42,47,74,87,89,101,112] The Gingival Index also has been documented as possessing interrater reliability.[1] These characteristics of validity and reliability are vital to the proper use of both the Gingival Index and the Plaque Index.

Home Care Instruction Effectiveness and the Proficiency of Scaling and Root Planing in the Removal of Calcified Bacterial Deposits

The goal of dentistry may be interpreted as oral disease examination and optimal natural dentition maintenance throughout an individual's lifetime.[77] Periodontal disease and its related bacterial plaque pose an active threat to this goal through the possibility of periodontal destruction. Scaling and supragingival plaque control, being a method of periodontopathic microflora reduction, can lead to an improvement of the periodontal status.[5,43,87,94,97] Through removal of bacterial inhabited plaque accumulations,

the source of destruction is reduced, thus producing an environment for periodontal improvement.

Several studies have been performed to investigate clinical and/or microbial effects which occur following the scaling and root planing of the dentition.[5,6,25,26,70,87,91,94,97,103] Hughes and Caffesse[26] investigated gingival positional changes (recession, reattachment, gingival width variations, pocket depth, mucogingival junction location) following scaling, root planing, and oral hygiene instructions in brushing and flossing. The total experimental sample, which contained 61 teeth of 15 patients, possessed labial (or buccal) inflammation extending into the attached gingiva. A total of three experimental sessions were performed in which the following indices and measurements were used to obtain clinical scores and measurements on the involved teeth: Inflammation Index, Kobayaski-Ash Plaque Score, clinical probing measurements, and bone presence underlying the mucogingival junction. One month following scaling, results revealed no significant changes in the location of the mucogingival junction. A one millimeter to two millimeter decrease in crevice depth was present for 45.9 percent of the cases. A one millimeter gain of new attachment was presented in 20.5 percent of the cases. The width of the keratinized gingiva decreased by one millimeter in 21.3 percent of the cases. The degree of plaque improvement was found to have no significant influence on any changes which occurred through the muco-

gingival junction, crevice depth, attachment level, gingival margin height, or keratinized gingival width. Clinical significance of this study indicated that scaling, root planing, and oral hygiene of teeth with severe gingival inflammation is followed by crevice depth decrease, attachment level gain, gingival recession, and decrease in the width of keratinized tissue within one week to one month after scaling. A decrease in inflammation yields results which indicate a tendency toward periodontal health.

Scaling and root planing, either with or without adjunctive tetracycline therapy, has been shown to qualitatively shift the subgingival microflora content.[26,87] A rapid reduction may occur in which a 10- to 100-fold decrease occurs during the initial two weeks of therapy (scaling, root planing, and oral hygiene instruction). A study conducted by Slots et al.[87] showed these results in five of six patients examined: four patients had moderate to severe adult periodontitis and two had localized moderate to severe juvenile periodontitis. Phase contrast microscopic analysis was performed utilizing samples from three test pockets of each subject. The dramatic decrease in microflora content was followed by a slow repopulation of approximately one-half the cell count during the following four to six months. Spirochetes also decreased following therapy and consequently increased to about one-third their original level after six months. Coccoidal forms and Actinomyces viscosus presented a percentage increase (10 to

40 percent) during therapy and then slowly reduced to their original proportions in 4 to 24 weeks. Cultivable gram negative anaerobic organisms and gram negative motile rods tended to decrease by 50 to 95 percent two to three weeks after therapy. The gram negative forms increased for approximately 16 weeks until reaching a peak, then reducing to pretreatment levels once again. Probing depth measurements, Gingival Index scores, Plaque Index scores, suppuration, interproximal alveolar bony changes evaluated by Björn measurements, and gingival fluid analyzations using a Harco meter, along with microbiological measurements were performed. Measurements were taken twice prior to treatment and after therapy (scaling, root planing and, if indicated, tetracycline) at intervals of 1, 2, 4, 8, 12, 16, and 24 weeks. Standardized radiographs were taken prior to therapy, at 12 and 24 weeks. Initial examinations revealed that pocket depths greater than five millimeters ranged from 19 to 75 percent. A reduction of 1 to 4 mm occurred in 20 to 90 percent following treatment, with no pockets exhibiting greater probable depth than that initially recorded. The presence of gingival bleeding existed in 50 to 100 percent of the examined gingiva prior to treatment. Following therapy, low levels of gingival bleeding (20 to 30 percent) were maintained throughout the study period of six months for five of the six subjects. The one remaining subject showed a lasting gingival bleeding decrease only following tetracycline administration. Pretreatment levels

of supragingival plaque illustrated 60 to 90 percent detectable deposits on buccal, lingual, or interproximal surfaces for all patients. Within one week following therapy, detectable deposits decreased to below 40 percent for five out of six subjects. The sixth subject maintained an 80 to 90 percent coverage. Suppuration was present in all patients before therapy, and was eliminated in five out of the six patients after the second week of treatment. The sixth patient maintained one to four suppurative areas throughout the post treatment period. Björn measurements were performed on 18 selected defects plus an additional 15. Over the six month period, 70 percent of the pockets remained unchanged while 27 percent showed bone apposition. One pocket exhibited bone loss three months following therapy initiation prior to retreating to its baseline level at six months. The percentage of gingival units that exhibited bleeding upon probing and the average gingival fluid flow were positively correlated at 90 percent ($p<0.01$). Both the gingival fluid flow and gingival bleeding correlated significantly with the total pocket flora and spirochetes ($p<0.01$). No significant correlations existed among the gingival bleeding and gingival fluid flow measurements and the percentage of motile rods, cocci, cultivable anaerobes, or cultivable gram negative organisms.

These results demonstrate that significant and long-lasting changes in the subgingival microflora associated with periodontal disease can be achieved by a single course

of scaling, root planing, and possible adjunctive tetracycline therapy. Subgingival organisms decreased 10- to 100-fold following initial therapy, with a 3- to 4-fold decrease in gram negative and anaerobic organisms. Following treatment, a microflora population predominated by the species of Actinomyces and Streptococcus was present for most pockets. Slots et al.[87] proposed the following periodontal treatment model: conventional therapy inclusive of thorough scaling and root planing; monitoring of subgingival flora and of the clinical course; and antimicrobial therapy usage in refractory cases. This study, in coordination with the investigation by Hughes and Cafesse,[26] illustrated positive results in the periodontal health status as a result of scaling and root planing instrumentation. However, the validity of the investigation performed by Slots et al.[87] was threatened due to the limited number of subjects utilized.

The response of periodontal pockets to root planing and/or oral hygiene was investigated by Tagge et al.,[97] while the response to root planing was studied by Proyle et al.[70] Tagge et al.[97] evaluated the soft tissue response of suprabony periodontal pockets treated by root planing and oral hygiene or by oral hygiene measures alone. Evaluation was performed by clinical measures (gingival examination, pocket depth, attachment loss, plaque index) and microscopic inflammatory measurement of gingival biopsies. Twenty-two patients with a mean age of 42.7 years participated. Three

selected pockets for each patient were studied: one control; one treated by oral hygiene procedures alone; and one treated by root planing and oral hygiene procedures. Each type of therapy produced a statistically significant reduction from the mean pretreatment score of gingival inflammation. A significantly greater reduction in inflammation was produced by the combined procedure of root planing and oral hygiene as compared to oral hygiene alone ($p<0.001$ clinically, $p<0.05$ histologically). Soft tissue pocket depth illustrated a decrease for both experimental treatments. A decrease from 3.0 mm preoperatively to 1.66 mm eight weeks postoperatively was observed for pockets treated by root planing and oral hygiene. This decrease was found, through a t-test for independent samples, to be significantly greater than the 2.86 mm preoperative and 2.32 mm postoperative difference shown by oral hygiene measures alone ($p<0.001$). Attachment loss in areas that received both root planing and oral hygiene was statistically significant, showing a 3.57 mm pretreatment to 3.05 mm post-treatment reduction as measured from the cementoenamel junction. A greater amount of plaque was found following eight weeks of treatment on surfaces that did not receive instrumentation and were grossly roughened in nature. Preoperatively, a dense infiltrate of chronic inflammatory cells was microscopically found to have replaced over half of the gingival fibers. (Microscopic and clinical gingival mean scores above five were present.) The gingiva was

edematous and bled easily. Following oral hygiene measures alone, both mean scores were decreased to approximately 3.8. The gingiva then exhibited slight marginal edema, delayed bleeding on probing, and a bank of chronic inflammatory cells. Through root planing and oral hygiene measures, chronic inflammatory cells were sparse and gingival appearance was normal with no bleeding present upon probing. The mean microscopic and clinical scores were 2.34 and 2.0, respectively. Results showed that eight to nine weeks following instruction and root planing, the incidence and severity of gingival inflammation were markedly reduced from pretreatment levels. Although oral hygiene measures alone reduced the mean pocket depth and incidence and severity of gingivitis, root planing followed by oral hygiene measures resulted in a statistically greater improvement of reduction of pocket depth and gingivitis severity and incidence.

Investigation by Proyle et al.[70] found favorable response of periodontal pockets to a single episode of root planing, through utilization of controlled probing forces. A total of 128 pockets exhibiting three to seven millimeters were used in this study. Pocket depth readings were taken immediately before a single episode of subgingival root planing and at one, two, three, and four week intervals following instrumentation. Oral hygiene instruction of plaque removal with multitufted and single tufted toothbrushes, interdental brushes, and Stimudents,[R] and a supragingival prophylaxis were performed at each time point. All

clinical parameters measured illustrated improvement over their baseline values. Parameters included the following: pocket depth and bleeding after manual probing and controlled probing force of 15 gm, 25 gm, and 50 gm; gingival margin location; attachment loss; and gingival and plaque indices. Pocket depth was significantly reduced for all probing forces one week (with significant gingival recession) and again three weeks (with significant gain of clinical attachment) following instrumentation. Bleeding was greatly reduced and virtually absent following three weeks. No control group was included due to literature that previously has documented investigations regarding such manipulations. Lack of a control group presented a threat to the validity of the investigation. Proyle et al.[70] concluded that substantial pocket depth reduction occurred within three weeks of a single episode of root planing due to initial gingival recession and secondary clinical attachment gain. This conclusion led to the justifiable decision that the biphasic response of gingival recession and coronal gain in clinical attachment was due to a combination of improved oral hygiene in addition to the single root planing episode. These results produced a cumulative value with those obtained previously which favored the combination of oral hygiene instructions and instrumentation for the reduction of gingival inflammation and pocket depth and promotion of oral health.[97]

Retardation of the development and progression of

gingival inflammation and destructive periodontal disease after an initial prophylaxis was investigated in a three-year longitudinal study by Suomi et al.[94] Two main groups, one experimental and one control, each of which consisted of 163 subjects, and three small study groups of 53 members each, were used. All experimental and control subjects were given a thorough dental prophylaxis. Within 3 to 14 days following the prophylaxis, baseline examinations were made, thus assuring a score of zero for subgingival and supragingival calculus. Baseline procedures included oral photography, radiographs, and indices for gingival inflammation, oral hygiene, and epithelial attachment level. Gingival inflammation was examined on eight selected teeth according to the Dental Health Center Index (DHCI). Oral hygiene was assessed through a modification of the Simplified Oral Hygiene Index (OHI-S). The Periodontal Disease Index (PDI) was the method utilized for measurement of the gingival inflammation and epithelial attachment level. Data was collected on experimental and control groups at one, two, and three-year intervals. The first-year reexamination occurred three months following a prophylaxis and excluded radiographs and pocket measurements. The second and third-year follow-up examinations included all parameters and took place two months after the prophylaxis. Control group members were involved only in the annual examinations and instructed to continue their routine home care. Experimental group participants were given repeated

instruction concerning oral hygiene care and periodontal disease in group sessions and individually. Included in the oral hygiene instruction were the following: personal oral hygiene care record, roll-stroke toothbrushing method, flossing, and disclosing tablet usage. A prophylaxis immediately followed the instruction. Experimental subjects received professional oral prophylaxes at two, four, six, and nine months the first year; at three-month intervals the second year; and at four-month intervals the third year. Additionally, these participants viewed their own oral debris through the use of phase microscopy. The experimental group's oral hygiene status was maintained at a maximum level of health.

Results showed an increased debris score in all groups above the baseline score. Both supra- and sub-gingival calculus scores were greater in the control group than the experimental group, as were gingival inflammation (DHCI and PDI) scores. Attachment loss in the control group was approximately 3.5 times greater than that of the experimental group. These findings provided evidence that strongly supported the development of programs on instruction of oral hygiene care and information for the control of periodontal disease.

The effect of supragingival plaque removal on anaerobic bacteria in deep periodontal pockets was investigated by Smulow et al.[89] Fourteen subjects who possessed at least four periodontal pockets with minimum depths of five milli-

meters participated. No professional oral prophylaxis had been performed for at least six months. Gingival Index, Plaque Index, and probing took place on days 1 and 21. Each pocket received random assignment to one of four study groups, three with treatment, one without treatment: (1) initial scaling and daily polishing, (2) daily polishing without initial scaling, (3) initial scaling without daily polishing, and (4) no treatment. Prior to sampling with a curet, supragingival deposits were reduced through use of gauze. The sample was immediately placed in 0.5 ml of reduced peptone yeast glucose broth medium, shaken to remove from the curet, and transported to an anaerobic chamber. Enriched blood plates, a nonspecific medium, were used to subculture anaerobic colonies to determine if an obligatory or facultative anaerobic condition existed. Gram stains and motility tests were performed.

Gingival and Plaque Indices reduced from 1 to 3 prior to treatment and 0 to 3 following treatment. This reduction was observed most consistently around teeth polished daily with or without subgingival scaling. Initial probing depths ranged from five to ten millimeters. Group 1 showed pocket reduction of 7 to 4 mm in ten pockets after treatment. Group 2 showed a 1 to 3 mm reduction in 11 pockets, while Group 3 showed a reduction of 1 or 2 mm in seven of the pockets. In group 3, a reduction of 1 mm was present in six pockets, while an increase of 1 mm was also seen in six pockets. The number of spirochetes present in

the periodontal pockets differed for all groups between the pre- and post-treatment levels. Groups 1 and 2 revealed statistically significant reductions in spirochetes while Groups 3 and 4 showed reductions, but were not significant in nature. Bacterial colonies grown on the nonspecific medium included gram-positive and gram-negative cocci and bacilli. Obligatory anaerobes grown included pigmented and/or nonpigmented colonies of nonmotile gram-negative small rods consistent with Bacteroides species. Mean reduction in colony forming units were statistically significant for Groups 1 and 2, but not for Groups 3 and 4. The kanamycin-vancomycin plates contained pigmented and nonpigmented nonmotile gram negative rods diagnosed as Bacteroides species. The results were statistically significant once again for Groups 1 and 2, but not for Groups 3 and 4. The number of organisms were reduced significantly in three weeks by daily removal of only supragingival deposits. No significant difference existed in the pockets that were and were not initially scaled, as long as daily removal of supragingival deposits occurred. The group that was not provided with daily removal of supragingival deposits, despite initial scaling and curettage, provided no significant reduction of bacterial components. Results provided by this group were not different from those shown by the control group. This evidence revealed the critical aspect of conscientious daily cleaning for eventual beneficial subgingival plaque results.

Stahl et al.[91] examined the healing of soft tissue following subgingival curettage and root planing. A total of 80 suprabony pockets, with a mean depth of 4.6 mm ± 0.9 mm were utilized. Adjacent facial or lingual pockets of similar clinical depth were chosen in each subject. One of these pockets was instrumented with Gracey curettes, including curettage and root planing extending to the clinical base. Following curettage, the adjacent nontreated gingival pocket was excised and histologically prepared, which served as the control specimen. Gingiva was removed at varying time intervals over an eight-week period. All 60 subjects were instructed in home care procedures. Control specimens demonstrated varying degrees of inflammation (23 mild, 28 moderate, 9 severe), as was shown through histologic analysis. Vascularity and degenerative changes in the connective tissue increased in association with inflammatory increase. Fifty-one control specimens showed a parakeratinized surface, while nine demonstrated facial or lingual keratinization. Fourteen specimens were observed histologically immediately following curettage. Fifty percent were found to contain disrupted epithelial lining while 50 percent had no epithelial lining. Inflammation varying from mild to severe (7 mild, 5 moderate, 2 severe) was present adjacent to the crevicular epithelium in all samples. Cinical pocket depth did not correlate with the inflammatory degree. Twelve specimens were histologically evaluated one week post-curettage. An intact crevicular

epithelial lining was present in all specimens. Inflammation varied in intensity (6 mild, 6 moderate, 3 severe). Parakeratinization was predominant at facial or lingual surfaces. The 14 specimens which were observed two weeks after curettage revealed epithelialization. Five specimens presented mild inflammation, while seven showed moderate and two severe; two samples demonstrated facial or lingual keratinization. Four weeks after curettage, eight of the 14 specimens taken revealed mild inflammation, while five showed moderate and one severe. Six of the 14 specimens analyzed six weeks after curettage presented mild inflammation, while five were moderate and three severe. At eight weeks postcurettage, mild inflammation was shown by six of the 12 examined specimens, while moderate inflammation was shown by three and severe by three. Due to the fact that a variation existed in inflammatory degrees in the evaluation of curetted and adjacent noncuretted pocket walls, the use of an adjacent margin (or papilla) as a control site was considered when inflammatory responses were evaluated. Histologic responses illustrated the following: (1) depth of inflammation did not correlate well with pocket depth and (2) degree of inflammation at one site could not predict the same at an adjacent soft tissue site.

The post-operative inflammatory response was analyzed with three statistical models. The first model tested shifts in before and after distributions and was concerned with group responses. The second model tested shifts from a

baseline inflammatory level. The third model tested shifts only within the individual. Significant changes with length of recovery time were revealed by the second model. This second model was consistent with the assumption that individual specimens from the same host are independent and reflective of an unchanging inflammatory response given normal metabolic variation. This led to the conclusion that inflammatory response at each tooth may be controlled primarily by local factors.

Axelsson and Lindhe[6] investigated the significance of maintenance care in the periodontal status of 90 patients who had been referred to the care of general practitioners following the end of active periodontal treatment. Treatment consisted of several factors: initial examination (assessment of oral hygiene, gingivitis, probing depths, attachment levels); individual instruction in proper tooth-cleaning methods; scaling; and modified Widman surgical technique. Throughout the first two months following surgery, patients were recalled once every two weeks for professional prophylaxis. At the end of this time period, reexamination of patients provided baseline data. Every third patient was referred (non-recall group) to the care of a general dentist with written information for regular maintenance care evaluations of oral hygiene, calculus formation, gingival conditions, and probing depth. A carefully designed and controlled maintenance care program was utilized by the remaining patients at the University of

Gothenburg School of Dentistry (recall group). This program involved two to three month recalls of instruction and practice in oral hygiene, meticulous scaling, and professional prophylaxis. All patients were reexamined three and six years following baseline examination. Oral hygiene conditions presented a marked improvement from the initial to the baseline examinations for both groups. Results revealed that those patients with the carefully designed recall program were able to maintain excellent oral hygiene standards and unaltered attachment levels over a six-year period. If not maintained in a supervised program as was the recall group, obvious signs of recurrent periodontitis were apparent at follow-up examinations. Results of this study present supporting evidence to previous studies by Stahl et al.[91] and Hill et al.[25] Conclusive results have indicated that removal of bacterial deposits is a necessity for initial periodontal therapy and the maintenance of periodontal health.

Additional evidence of the importance of plaque control was presented in an investigation performed by Tabita et al.[96] Supragingival plaque control, sub gingival plaque development, and their effects on gingival health were evaluated. Twelve patients with pocket depths of 4 to 6 mm were utilized. Gingival inflammation was assessed initially and following the 14-day experimental period, by the Löe and Silness Index. Thorough scaling, root planing, and polishing were performed on three out of the four quadrants

in each subject. Following this prophylaxis, one of three plaque control modalities were assigned to each quadrant: (1) daily professional supragingival plaque removal, (2) instructions for specific brushing and flossing by the patient, or (3) no plaque control method. All initially untreated fourth quadrants were divided equally into three groups and correspondingly assigned one of the plaque control modalities. Results showed that bacterial plaque reforms subgingivally 14 days after scaling, root planing, and polishing regardless of effective supragingival plaque control. The degree of gingival inflammation was improved significantly by removal of supragingival plaque control. When quadrants, beginning with and without scaling and root planing instrumentation, that had received supragingival plaque control were compared after 14 days, no significant difference between gingival scores was found. The benefits of scaling and root planing are of short duration because of subgingival plaque reformation. These findings indicate contributions of both supra- and sub-gingival plaque removal to the clinical gingival appearance. Pockets four to six millimeters in depth present a situation not possible to control through scaling, root planing, and supragingival plaque control. The removal of all plaque and calculus, both supra- and sub-gingival in location, must be performed for total plaque control therapy.

Currently there exist many forms of periodontal therapy, including scaling and root planing, oral hygiene,

and subgingival curettage.[5,6,25,26,70,87,91,94,97] No individual treatment may be claimed as a sole periodontal therapeutic method independent of other methodologies. The combination of scaling, root planing, and oral hygiene has been shown to be beneficial.[26,70,87,94,97] Beneficial aspects of surgical procedures is dependent upon the removal of bacterial deposits and plaque which serve as irritants to the adjacent gingival tissue.[5,6,25,91] Optimal periodontal results that have been obtained in investigations have included oral hygiene instructions as an integral part of therapy.[5,6,25,26,70,87,91,94] Treatment methodologies utilized in any given situation will be dependent upon the therapist and individual conditions present. However, whatever the optimum treatment decision, individual oral hygiene instructions must be presented and adhered to closely to prevent the recurrence of established bacterial plaque. Inclusion of optimum oral hygiene maintenance to the treatment methodology prescribed will allow interruption and prevention of the pathogenic sequence of periodontal disease.

The Specific Plaque Hypothesis in Relation to Periodontal Disease Treatment and Occurrence

An essential component of periodontal disease initiation and progression is bacterial plaque.[4,16,18,32,33,41,42,49,75,82,84,99] Loesche[49] has developed two plaque hypotheses describing this phenomenon. The first of these hypotheses states that all bacteria found in plaque contri-

bute to the occurrence of periodontal disease.[32,49] This is known as the nonspecific plaque hypothesis (NSPH) and directs treatment of dental diseases toward the elimination or suppression of all plaque bacteria. The NSPH omits the necessity of bacteriological diagnosis. This hypothesis has led to the development of treatment strategies that are dependent upon mechanical procedures for the control of plaque accumulations. The safety of mechanical procedures cannot be paralleled by daily utilization of chemotherapeutic agents for plaque control, although chemotherapeutic agent usage is possible with the NSPH.

The second hypothesis proposed by Loesche[49] is the specific plaque hypothesis (SPH). The SPH states that periodontal disease is caused by a specific bacterial infection. This hypothesis supports recent observations that conclude specific microbial complexes to be associated with periodontal disease.[4,16,18,32,33,41,42,75,82,84,99] Through the specific plaque hypothesis, treatment strategies may be formulated utilizing chemotherapeutic agents.[49] The choice of agents will be based upon the diagnosis of precise clinical and bacteriological criteria that will identify the plaque as odontopathic, which is not possible with the NSPH. The time of treatment termination is determined through clinical healing and/or odontopathic flora reduction. Improvement that results from treatment will be produced through repopulation of dentogingival surfaces by a nondisease-associated flora when treatment is halted.

Chemotherapeutic agents should be broad spectrum antimicrobial in nature due to the complexity of the periodontopathic flora. Chlorhexidine and fluorides are two examples of broad spectrum agents. The agent does not need to be delivered to the entire mouth, only to dentogingival surfaces (the site of the infection). A short-term usage of the antimicrobial agent is determined by the specific plaque hypothesis. The strategy used consists of disruption of existing disease flora to proportions so low as to disadvantage their recolonization process. This disruption permits the reestablishment of a different nonpathogenic flora. Eventual length of treatment and dosage levels must be determined to establish ultimate benefit-to-risk ratios associated with clinical improvement.

A rationale designed to improve the management of any therapeutic regimen for the control and prevention of periodontal disease has been described by Keyes[29] as microbiologically modulated periodontal therapeutics (MMPT). This rationale is based upon the specific plaque hypothesis.[49] The government of creviculoradicular plaque infections in the human dentition through MMPT may be incorporated into management of any treatment regimen.[29] The use of microbiological monitoring in periodontal therapy management adapts a long-established medical rationale for the diagnosis and treatment of creviculoradicular infections. Through microscopic use, the prognosis greatly may be improved or no influence may occur. Micro-

biologically modulated periodontal therapeutics maintains that bacterial risk factors associated with periodontal diseases will be identified and become therapeutic targets. Appropriate antibacterial measures then must be tested to determine effectiveness and will be modified when necessary. Bacterial risk factors associated with periodontal lesions cannot be adequately assessed by clinical or microscopic examinations or tactile sensations. Once diagnosed, therapeutic measures are employed with the ultimate goal of conversion from disease-associated bacterial complexes to health-associated populations. This goal parallels that of Loesche's specific plaque hypothesis.[49] Investigating the specific microbiota B. melaninogenicus may aid in diagnosis and decrease of bacterial risk factors associated with periodontal disease.

Three Percent Hydrogen Perioxide, Sodium Chloride, and Sodium Bicarbonate as Chemotherapeutic Agents in the Treatment of Periodontal Disease

Chemotherapeutic agents such as hydrogen peroxide, sodium bicarbonate, and sodium chloride in combination, alone, or with water have been utilized in studies for individual home care treatment of periodontal disease.[9,30,32,34,61,78,79,110,112] Keyes et al.[30,32,34] utilized chemotherapeutic agents in a nonsurgical periodontal disease treatment regimen, based upon the specific plaque hypothesis. In 1978, the nonsurgical treatment was reported to consist of utilization of the following: (1) sodium

bicarbonate and hydrogen peroxide, with or without table salt, as a dentifrice applied through sulcular brushing and flossing; (2) preliminary calculus removal and curettage; and (3) systemic tetracycline.[33,34] Disease activity or remission was monitored through utilization of gingival bleeding points and phase-contrast microscopy for sixteen bacterial forms and white blood cells.[33] A basic oral hygiene regimen was presented to each patient for the promotion of effective daily removal of accumulated bacteria and toxic by-products. Toothbrushing with a sulcus brush in a vibratory manner and flossing were the first steps. This was followed by utilization of the therapeutic adjuvants sodium bicarbonate, 3% hydrogen peroxide, and in some instances, sodium chloride (or magnesium sulfate if sodium chloride could not be used due to medical considerations). (Specific criteria for the inclusion of sodium chloride was not specified.) This mixture was applied through use of the toothbrush, floss, or toothpicks. This was followed by irrigation either with water or a warm saturated salt solution. If root sensitivity occurred, sodium fluoride was applied at bedtime. Phase contrast microscopy was utilized to monitor the therapeutic regimen's effectiveness through designated subgingival plaque specimens. The following microbial types were recorded: white blood cells, small and large motile cocci, motile rods, thin and thick flexing rods, spiral rods, "arm-clocks," spirochetes, brush configurations, trichomonas, amoeba, and others. Adjustment

of therapeutic intensity was in accordance with recorded periodontopathic bacteria and white blood cells. If patients continued to demonstrate large numbers of motile bacteria and white blood cells in residual pocket spaces following prescribed antibacterial therapy and preliminary calculus removal and curettage, a course of tetracycline hydrochloride, 250 mg four times a day, was provided for two weeks. If microscopic fields remained positive following two weeks of antibiotic therapy, the tetracycline was continued for an additional week.

The following results were observed in patients who were instructed to diligently follow prescribed home care regimens:[34] (1) Spirochete and large motile rod populations decreased to non-detectable levels; white blood cell counts decreased as low as five per field; (2) rapid reduction occurred in gingival inflammation, bleeding, and suppuration; (3) teeth became less mobile; (4) resorption of bone ceased or abated to imperceptible degrees; and (5) pocket depths were similar or less as compared to baseline figures six months to one year later. Findings showed that applications of saturated salt solutions, as prescribed, into sulcus-pocket areas; and, if indicated, periodic courses of systemic tetracycline can markedly reduce potentially periodontopathic bacterial populations. The extent to which the results may be related to situations outside the laboratory setting are minimal due to the questionable validity of the study. Several factors existed that threatened the investi-

gation's validity: no designation of experimental and control groups; limited sample population; and lack of published scientific data and statistical analysis, documented intra- and inter-rater reliability, and an overall valid research design.

The effect of Keyes' method of oral hygiene (including baking soda, hydrogen peroxide, and salt water) as an alternative to traditional periodontal therapy was investigated by Greenwell et al.[115] Oral hygiene, gingival conditions, subgingival microbial proportions, probing depths, and bleeding on probing were measured to ultimately determine the presence or absence of a subgingival microflora compatible with periodontal health. Two groups, each consisting of nine patients, were selected in accordance with set criteria. All subjects in Group I had moderate to severe periodontitis and a minimum of two pairs of contralateral teeth with pocket depths of 5 mm or greater and 25% alveolar bone loss. Group II consisted of subjects with pocket depths of less than 5 mm that had received periodontal surgical therapy within four years. (The pocket depths were 5 mm or greater prior to the periodontal surgery). All subjects possessed a minimum of 20 natural teeth, had received no oral prophylaxis or antibiotics within six months, and had no systemic disease history. A split mouth experimental design was used allowing the Keyes' method and conventional oral hygiene to be randomly assigned to opposite sides of the oral cavity. One arch received

scaling and root planing while the opposing arched received no instrumentation. Data collection occurred for one tooth in each quadrant.

The Plaque Index, Gingival Index, gingival fluid flow, and darkfield microscopic examination of subgingival plaque were measured at 0, 2, 4, 6, and 8 weeks. Probing depths were recorded initially and at the conclusion of the study. Bleeding on probing was recorded at week 8. Patients were instructed in the Keyes' method of oral hygiene (experimental side) and in the conventional method (control side) which consisted of flossing and brushing with a circular scrub technique. A sterile curet was utilized to collect subgingival microbial samples from the deepest accessible subgingival zones. Coccoid cells, others (straight rods, filaments, fusiforms), motile rods, and spirochetes were recorded. A separate three-way and four-way mixed analysis of variance was the statistical method used.

Results varied for the scaled and unscaled arches and for areas that had and had not received previous surgical procedures. The unscaled arch receiving the Keyes' method resulted in significantly better Plaque Index and Gingival Index at times 4 and 8 ($p<0.01$), and significantly better gingival fluid flow at time 4 ($p<0.01$) and time 8 ($p<0.05$). Surgical procedure status had a highly significant influence ($p<0.001$) for the Plaque Index at 0 and 4 weeks, the Gingival Index at 4 and 8 weeks, and the gingival fluid at 0 weeks. Arches receiving instrumentation provided no statis-

tically significant differences ($p<0.05$) at anytime between experimental and control sides.

Microbiological indicaters also revealed various data concerning the testing sites. Week 0 presented no significant differences. The experimental side revealed higher proportions of coccoid cells ($p<0.01$) and lower proportions of spirochetes at time 4 ($p<0.01$) and 8 ($p<0.05$). Motile rods and spirochetes were significantly lower at time 8 ($p<0.05$) on the experimental side. Bacterial proportions were significantly more favorable at all times (0, 4, and 8) in the group that received surgery. Prior to instrumentation, no differences in morphotypes between the control and experimental sides were present. Increased favorable proportions of all categories were found following scaling. Surgery again was associated with more favorable proportions of coccoid cells and motile rods ($p<0.05$) and spirochetes ($p<0.01$).

Results indicated that pathogenic subgingival microflora was not highly controlled by oral hygiene alone. Scaling and root planing instrumentation were responsible factors in the primary observed antimicrobial effect.

Sodium bicarbonate and hydrogen peroxide have been dually studied concerning their effectiveness in the treatment of periodontal disease.[9,110] West and King[110] attempted to isolate and test the therapeutic effectiveness of toothbrushing with hydrogen peroxide-sodium bicarbonate, supplemented with scaling and systemic antibiotics. A total

of 42 subjects were selected for pocket suppuration and divided into two groups. Group I, which consisted of 22 subjects, alternately was assigned to experimental or control groups. Patients were treated sequentially with brushing instructions, scaling (ultrasonic and hand instrumentation), and systemic antibiotics. (The sequential treatment allowed the brushing therapy to be studied for 28 days prior to scaling.) Experimental subjects used the 3% hydrogen peroxide-sodium bicarbonate dentifrice, while control subjects used a commercial sweetened and flavored calcium carbonate tooth powder mixed with tap water. The dentifrices were forced subgingivally with a soft nylon toothbrush and unwaxed dental floss. Group II consisted of 20 subjects who were assigned to experimental and control groups. Group II was treated concurrently (not sequentially) with brushing and root scaling. No systemic antibiotics were administered to this second group. Subjects were monitored by darkfield microscopy for motile rods and spirochetes and examined for suppuration at 14-day intervals for seven weeks. Results indicated an approximate 33% reduction in suppuration sites for both dentifrices involved. No differences between experimental and control subgroups and no significant changes in darkfield counts existed. Scaling, either concurrent (Group II) or subsequent (Group I) to toothbrushing illustrated a statistically significant reduction (approximately 70%) in the number of suppuration sites. Suppuration counts one week

after antibiotics yielded statistically identical results for both experimental and control groups. No detectable differences were found to exist among the two dentifrice groups. The investigation to be performed also will view two dentifrices (one experimental and one control). The effect of scaling in addition to the dentifrices also will be researched.

Another study performed compared a sodium bicarbonate-hydrogen peroxide dentifrice to a fluoridated paste.[9] Cerra and Killoy[9] utilized four patients with periodontal pockets of four to seven millimeters, through a split mouth design. Each patient was instructed to brush assigned quadrants with a sodium bicarbonate-3% hydrogen peroxide paste using sulcular brushing and a Perio Aid^{R}. Fluoridated paste was utilized in the same manner upon the control sides. Darkfield microscopy was utilized on days 1 and 21 to examine microbial flora of pocket depths recorded at the corresponding time. Cell morphology and bacterial motility were recorded. No significant differences were found to exist between the medicament and control sites for the microbial flora at the 0.005 level. Probing depths were reduced similarly on both sites. The researchers concluded that sodium bicarbonate and hydrogen peroxide have no unusual benefitts in the reduction of periodontopathic microflora. Validity of the study was threatened due to the small number of subjects (four) and limited time schedule (21 days) involved.

Saturated salt solutions, especially sodium chloride, may have therapeutic value in the treatment of periodontal disease.[61] Sodium chloride has been investigated for its possible beneficial effects in treatment.[61,112] Wolff et al.[112] compared the effect of phase-contrast microscopy combined with topical antimicrobial use of sodium chloride and hydrogen peroxide to conventional home therapy (dental floss, non-fluoridated toothpaste) for control of the plaque microbiota and periodontal inflammation. In both groups, a reduction in motile bacteria occurred at four weeks, while spirochetes reduced in numbers at four through sixteen weeks. All clinical parameters evaluated, which included the Silness and Löe Plaque Index, Löe and Silness Gingival Index, Muhlemann and Son Bleeding Index, and pocket depth, were decreased at four weeks and maintained through sixteen weeks. The sodium chloride and hydrogen peroxide regimen, as compared to the conventional regimen, was statistically greater in reduction of pockets greater than or equal to four millimeters as seen up to eight weeks. No significant differences were observed in relation to plaque, bleeding, or gingival indices. In pockets less than or equal to three millimeters, the two regimens paralleled on microbiological and clinical parameters. However, prior to eight weeks, pockets greater than or equal to four millimeters presented a reduction, though not statistically significant in motile bacteria and pocket depth with the sodium chloride and hydrogen peroxide regimen.

The utilization of an effective oral hygiene home care

regimen was discussed previously as being a valuable tool in the prevention and treatment of periodontal disease.[5,6,25,26,70,87,91,94,97] Investigations have been conducted concerning the chemotherapeutic use of hydrogen peroxide, sodium bicarbonate, and sodium chloride either in combination, alone, or in conjunction with water.[9,30,32,34,61,78,79,110,112] Studies performed by Keyes et al.[30,32,34] involved the utilization of these chemotherapeutic agents under rationale provided by the specific plaque hypothesis.[29,49] Results showed an improvement in the oral health of the subjects, [33-34] which coincide with results illustrated by West and King[110] and Wolff et al.[112]

Although gingival and periodontal health were recorded as clinically and microscopically improved over baseline conditions, the actual benefits of the use of various combinations of hydrogen peroxide, sodium bicarbonate, and sodium chloride indicate conflicting conclusions. While some studies concluded no additional benefits of the agents when compared to toothpowder or a fluoridated dentifrice,[9,110] others concluded advantageous results above the conventional therapeutic regimens.[33,34,112]

Further research is needed in the comparison of these chemotherapeutic agents and conventional home care therapy. The comparison of the dentifrices as performed against both the scaling and nonscaling of the dentition is in need of further investigation. Through the microscopic assessment in coordination with the specific plaque hypothesis[49] and

clinical assessment of these areas, additional results may be achieved. Perhaps these additional results will enable an increase in the dental profession's knowledge of the prevention and treatment of periodontal disease.

Summary

The dental profession is one which is responsible for maintenance of the dentition and oral cavity in an optimum state of health. Periodontal disease poses a threat to this healthy condition and if allowed to continue, may result in complete destruction of the periodontium. Various treatments available for periodontal disease currently are being utilized; however, no single treatment may be said to possess significant advantage over another.

Microbiota and associated evaluation are a means for monitoring periodontal disease progress. Through initial bacterial assessment and continued observation, the microbial status may be followed and evaluated. If one accepts the specific plaque hypothesis by Loesche,[49] monitoring subgingival bacteria may be an irreplaceable tool in periodontal disease treatment.[32] Studies that have been conducted generally agree upon the concept of bacteria associated with periodontal disease.[4,16,18,32,33,41,42,75,82,84,99] However, there exists a need for further studies concerning specific microbial association and treatment prior to positive conclusions.

Scaling and root planing present as being beneficial in associated clinical and antimicrobial effects, as does oral

hygiene. A combination of these methodologies present cumulative benefits to periodontal health.[26,70,87,94,97] Inflammatory conditions affected by scaling, root planing, and oral hygiene result in a decrease in crevice depth, a gain in attachment level, a decrease in the width of keratinized tissue, and/or recession of the gingiva. The advantageous results associated with scaling and root planing may be realized following instrumentation due to the removal of bacterial deposits that serve as irritants to the periodontal tissues.[5,6,25,91]

The utilization of antimicrobial agents in the treatment of periodontal disease correlates with the specific plaque hypothesis.[29,49] Advantages and disadvantages have been shown for combinations of sodium chloride and hydrogen peroxide; sodium bicarbonate and hydrogen peroxide; and sodium chloride, sodium bicarbonate, and hydrogen peroxide.[9,33,34,110,112] The dentifrice comprised of sodium chloride, sodium bicarbonate, and hydrogen peroxide, which will be utilized in this study, has been shown to be beneficial in combination with other clinical parameters.[33,34,112] Further research is needed in the area of antimicrobial agent usage utilizing valid and long-term studies. The comparison of these chemotherapeutic dentifrices, as performed against both scaling and nonscaling of the dentition, is also in need of investigation. Further investigation will enable one to more fully understand the role of chemotherapeutic agents in

the periodontal health of the oral cavity.

The threat periodontal disease poses upon the dental health of every individual is one which must be controlled. Various treatments for this destructive disease exist, each possessing its own advantages and disadvantages. Although this investigation must be limited to a short-term status, the treatments need further long term research in order to provide definitive information that can be generalized to other populations.

CHAPTER 3

Methods And Materials

A comparison of the effectiveness of a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice to a distilled water, sodium bicarbonate dentifrice on anaerobic sulcular microbiota, gingival health, and plaque accumulation was investigated. The dentifrices were utilized once daily in quadrants which had and had not been scaled and root planed. The split mouth experimental design was employed. Microbial, gingival, and plaque measurements through use of plated culture media, the Gingival Index, and the Plaque Index were performed at four-week intervals throughout an eight-week period. Home care regimens were reviewed at two-week intervals with week six being optional. The Hampton Veteran's Administration Medical Center Dental Clinic was the location of the investigation.

Sample Description

Subjects who participated in the study were registered patients at the Hampton Veteran's Administration Medical Center. A total of twenty male subjects from this population were chosen in accordance with set criteria to participate. The sample size was limited to twenty due to time and resource limitations. Initial screening of the

patients was performed by the principal investigator in the dental clinic. Five subjects voluntarily withdrew from the study thus leaving a sample size of fifteen.

Several characteristics composed the essential criteria met by the subjects during the initial screening. The subjects were 23 to 64 years of age and possessed a minimum of twenty natural teeth, including at least two of the four teeth entitled experimental teeth (first premolar, second premolar, first molar, second molar) in each quadrant. Pocket depths of four to seven millimeters were present in a minimum of one location for each experimental tooth. An inflammatory state of mild to severe inflammation existed as shown through Gingival Index scores. Acceptable subjects were limited to medical-risk categories I or II as defined by the American Society of Anesthesiologists (A.S.A.).[54] Those subjects who possessed the following medical characteristics were excluded: uncontrolled hypertensive disorders; uncontrolled diabetes; seizure disorders controlled with dilantin, phenobarbital, or their derivatives; requirement of prophylactic antibiotic coverage (i.e., valvular prosthesis, hip prosthesis); sodium restricted diets; hepatitis; venereal disease; tuberculosis; acquired immune deficiency syndrome; active gastrointestinal disorders; history of radiation to the head and neck region; history of steroid therapy within one year of initiation of the study for a minimum two-week time period; compromised immune system; Hodgkin's disease; chemotherapy; and immuno-

suppressive agents. The subject must not have had antibiotic coverage two months prior to the study. Also excluded were those with mental or physical impairments that interfered with the performance of proper oral hygiene procedures and those who had undergone periodontal surgery within the past year which involved direct incision with a periodontal blade.

Research Design

The 2x3 factorial research design associated with this investigation provided multinomial response factor measurements (Appendix A). This design was appropriate for the research topic since it provided results that yielded valid information to the dental profession concerning oral health care maintenance and periodontal disease treatment and prevention. The use of blind experimental procedures prevented the principal investigator from knowing which quadrants were designated as experimental or control.

A total of two independent variables were used in this investigation. The first was a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice versus a distilled water, sodium bicarbonate dentifrice. The second independent variable was periodontal instrumentation versus no periodontal instrumentation. Each subject randomly was assigned scaling and root planing for either the maxillary or mandibular arch (Appendix B). Instructions for home care procedures were followed by periodontal instrumentation.

Three dependent variables were involved in this study.

The first was culture media analysis measurement of the sulcular anaerobic microbial species Bacteroides melaninogenicus. Measurements took place initially (on the first day of the investigation), at four weeks, and at eight weeks for the time span of the study. Microbial samples were taken with a sterile explorer from the deepest pocket location found among the first molar and second premolar present in each quadrant. If one or both of these teeth was/were absent, the first premolar and/or second molar was/were utilized. If the initial pocket measurements recorded for any single tooth were numerically equivalent for all six areas (disto-buccal, buccal, mesio-buccal, disto-lingual, lingual, mesio-lingual), the buccal surface was the location of sampling. Supragingival plaque was reduced prior to instrumentation by wiping the surface with a sterile gauze square. The second dependent variable involved the Gingival Index. Measurements of the gingival inflammatory condition were taken immediately following the microbial sampling procedures. Four gingival areas (buccal, mesio-buccal, disto-buccal, lingual) for each experimental tooth were measured. The Plaque Index constituted the third dependent variable. Severity and location of soft debris aggregates of each experimental tooth were determined for two areas (buccal, lingual) for each experimental tooth prior to microbial sampling.

History and maturation were controlled by randomly assigning the unilateral quadrants as either experimental or

control. Periodontal instrumentation was assigned randomly to maxillary or mandibular arches of the oral cavity. Intrarater reliability was determined prior to conducting the investigation through test/retest procedures performed upon four patients not participating in the investigation.

Random assignment of the unilateral quadrants to one of two sides, experimental or control, allowed for control of subject selection bias and subject relevant variables. Mortality was controlled partially through use of patients from the Hampton Veteran's Administration Medical Center. Mortality that was caused by dismissal of patients from the facility or by personal decisions to cease participation could not be controlled in this investigation. Situation relevant variables were controlled for by the constant utilization of the same environment, time of day, and experimenters. The independent variable, scaling and root planing, was performed by the principal investigator.

Methodology

The investigation was conducted at the dental clinic in the Hampton Veteran's Administration Medical Center. Patients were examined initially by the principal investigator to determine if criteria for the study were present. Each patient was between the ages of twenty and seventy years and presented a minimum of twenty teeth. Among the twenty teeth, each quadrant contained at least two of the following experimental teeth: first premolar, second premolar, first molar, second molar. If all four experi-

mental teeth were present in any quadrant, the second premolar and first molar were studied. Pocket depths of the experimental teeth in each quadrant included measurements of four to seven millimeters for a minimum of one area for each tooth. Pocket depths did not exceed seven millimeters as measured by a color-coded probe with markings of 3-6-9-12. The periodontal probing technique used by the principal investigator was standardized with use of a test-retest method to determine intrarater reliability.

Inflammatory condition of the gingiva surrounding the experimental teeth involved mild to severe inflammatory status, as determined through the Gingival Index.[45] The gingival condition of four surfaces (buccal, mesio-buccal, disto-buccal, lingual) were examined for all experimental teeth. Surfaces assessed demonstrated measurements of either one, two, or three.

Patients chosen for participation in the study were in medical-risk categories I or II as defined by the American Society of Anesthesiologists (A.S.A.).[54] Those subjects who possessed the following medical characteristics were excluded: uncontrolled hypertensive disorders; uncontrolled diabetes; seizure disorders controlled with dilantin, phenobarbital, or their derivatives; requirement of prophylactic antibiotic coverage (i.e., valvular prosthesis, hip prosthesis); sodium restricted diets; hepatitis; venereal disease; tuberculosis; acquired immune deficiency syndrome; active gastrointestinal disorders; history of

radiation to head and neck region; history of steroid therapy within one year of initiation of the study for a minimum two-week time period; compromised immune system; Hodgkin's disease; chemotherapy; and immunosuppressive agents. The subject must not have had antibiotic coverage within two months of the study. Those with mental or physical impairments that interfered with the performance of proper oral hygiene procedures and those who had undergone periodontal surgery within the past year which involved direct incision with a periodontal blade were also excluded. Manual dexterity must have been present to a degree high enough that self-oral hygiene method could be accurately employed.

Fifteen subjects who met the designated criteria presented in the initial screening appointment were utilized in the experiment. Unilateral quadrants of each subject were assigned randomly to one of two sides (expermental or control) through the flip of a coin. Random assignment occurred for each arch, through the flip of a coin, to determine if the maxillary or mandibular portion received scaling and root planing instrumentation (Appendix B).

Initial experimental appointment procedures began with the subject signing an informed consent form explaining the purpose of the research, the rationale and importance of participation, and requirements to be followed (Appendices C and D). A complete medical history form soliciting the subject's past and present medical background was recorded

using the Hampton Veteran's Administration Medical Center Dental Clinic's form (Appendix E).

The gingival inflammatory condition was determined through the Gingival Index. Four surfaces (buccal, mesio-buccal, disto-buccal, lingual) for each experimental tooth were utilized. Following thorough drying of the gingival surface, the color coded probe was used to determine susceptibility to bleeding through probing and to palpate the facial gingival surface to determine the presence of edema. Inflammation was measured according to a numerical scale of 1, 2, or 3. The scale criteria ranged from mild to severe inflammation:

1 = Mild inflammation--slight change in color, slight edema.

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

3 = Severe inflammation--marked redness and edema. Ulceration. Tendency to spontaneous bleeding.[45]

Following the probing of the appropriate gingival portion, the numerical value corresponding to the inflammatory degree was recorded. All Gingival Index Scale values, including mild to severe inflammatory condition, could have been present (Appendices F and G).

The second dependent variable, the Plaque Index, was performed upon all experimental teeth in sequence with the Gingival Index. Two surfaces (buccal, lingual) were evaluated for each tooth visually and by running a probe across the appropriate tooth surface after drying with

air. A numerical scale of 1, 2, 3 was used. The scale criteria ranged from a film of plaque to an abundance of soft matter:

> 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.[45]

After examination of the soft debris deposit visually and with a color-coded probe, the numerical value that corresponded with the degree of accumulation was recorded (Appendices B and H). All values in the Plaque Index criteria range could have been observed and recorded.

A small subgingival microbial sample was obtained from the deepest periodontal pocket of the experimental teeth in each quadrant. A sterilized explorer was utilized to obtain each sample. Prior to sampling, the supragingival surfaces of experimental and adjacent teeth were carefully cleaned with sterilized two-by-two inch gauze squares in order for supragingival plaque contamination to be kept to a minimum. When obtaining the sample, the explorer was placed at the bottom of the periodontal pocket, and the tooth and tissue were simultaneously instrumented. A sample of plaque was defined as the amount of material that covered the end of the explorer. Pockets that did not yield the appropriate

amount of sample material during the first procedure underwent a second scraping. The sample was dispersed in a sterile tube containing 0.5 ml thioglycollate. Plated culture medium was then streaked, placed in an anaerobic jar, and incubated for a minimum period of seven days. Resulting black-pigmented colonies were viewed and counted through use of a fluorescent light. All counts were recorded (Appendix I). Anaerobic blood agar specific for the selection of Bacteroides melaninogenicus was utilized.

Following index recordings and microbial samplings, the designated quadrants for the subject present were scaled and root planed. All teeth in the designated unilateral quadrants were scaled and root planed with the use of Gracey curettes, universal curette, sickles, and files to remove all calcified deposits. Instrumentation was halted when no indications of deposit presence were detected with the CH3 (pigtail) and #17 (right angle) explorers.

The subject was then instructed in the performance of the appropriate home care regimen by the preventive therapist (Appendix J). Subjects were instructed in the proper use of unwaxed dental floss. The control dentifrice of distilled water and sodium bicarbonate then was mixed. One teaspoon distilled water was placed in a bowl, and the bristles of the toothbrush designated "water" moistened. Three and one-half level teaspoons of sodium bicarbonate were added to the distilled water and mixed with the teaspoon until a paste consistency was achieved. The paste

was smeared along the gingival margins of the maxillary quadrant on the control side with the toothbrush bristles, from the midline adjacent to the central incisor to the last posterior tooth. The use of a fine rubber tip stimulator was taught and utilized to incorporate the applied dentifrice into the gingival sulcus. Next, the modified Bass method of sulcular brushing was taught through use of a sulcus toothbrush. This brushing method also aided in sulcular incorporation of the dentifrice. The patient flossed the involved teeth. Instructions were given to continue with the mandibular quadrant in the same manner, followed by thorough rinsing.

The experimental dentifrice was mixed and applied in a similar manner. A separate mixing spoon and bowl were used. One-half teaspoon 3% hydrogen peroxide and one-half teaspoon distilled water were placed in a bowl and the bristles of the toothbrush designated "peroxide" moistened. One-fourth teaspoon sodium chloride and three and one-half teaspoons sodium bicarbonate were added to the 3% hydrogen peroxide and mixed with the teaspoon until a paste consistency was achieved. The remaining steps were performed in an identical manner as those for the control dentifrice of distilled water and sodium bicarbonate. The patient was instructed to continue the procedure for both quadrants, one at a time, in this manner. An information sheet containing prescribed oral hygiene home care regimens and mixing instructions for the pastes was given to the patient (Appendix K).

Following index recordings, microbial samplings, periodontal instrumentation, and home care instructions, an appointment time for the next session was scheduled, and the patient was dismissed. This first session lasted a maximum of two hours and thirty minutes.

The remaining two sessions at four-week intervals were conducted in the following manner. At each session, the medical history was updated to ensure no significant changes were present. Next, the microbial status, gingival condition, and plaque accumulation were evaluated through anaerobic culture media, Gingival Index, and Plaque Index procedures. The patient's oral hygiene home care regimen was evaluated at two-week intervals (week six was optional) by the preventive therapist to ensure proper technique utilization. The Personal Hygiene Performance (PHP) test was also performed. Necessary corrections were made concerning the home care procedures if appropriate. The patient was then dismissed. Maximum time allotted for each of these sessions was one hour.

All information obtained in the investigation remained completely confidential. All records containing subjects' names were destroyed at the conclusion of the study. Publications which may follow the study will include no procedures, results, or information that violate the confidentiality of the investigation.

Protection of Human Subjects

1. Subject Population. The subjects selected were

from among those currently registered at the Hampton Veteran's Administration Medical Center. A total of twenty subjects were chosen who met the following criteria: 23 to 64 years of age; male or female; minimum of 20 natural teeth, which included at least two of the four teeth entitled the experimental teeth (first premolar, second premolar, first molar, and second molar) in each quadrant; pocket depths of four to seven millimeters in a minimum of one location for each experimental tooth; and a mild to severely inflamed gingival condition surrounding the experimental teeth. Subjects were in the medical risk categories I or II as defined by the American Society of Anesthesiologists (A.S.A.). Those subjects possessing the following medical characteristics were excluded: uncontrolled hypertensive disorders; uncontrolled diabetes; seizure disorders controlled with dilantin, phenobarbital, or their derivatives; requirement of prophylactic antibiotic coverage (i.e., valvular prosthesis, hip prosthesis); sodium restricted diets; hepatitis; veneral disease; tuberculosis; acquired immune deficiency syndrome; active gastrointestinal disorders; history of radiation to the head and neck region; history of steroid therapy post January 1, 1984, for a minimum two-week time period; compromised immune system; Hodgkin's disease; chemotherapy; and immunosuppressive agents. The subject must not have had antibiotic coverage since November 1, 1984. Also excluded were those with mental or physical impairments that interfered with the

performance of proper oral hygiene procedures, and those who had undergone periodontal surgery which involved direct incision with a periodontal blade within the past year.

2. Consent Procedures. Informed consent of each subject was obtained at the beginning of the initial experimental appointment. Each subject was verbally instructed in the rationale of the experiment, the importance of their participation, and any risks or benefits that were involved (Appendix D). Documents stating their consent for involvement in the study as a subject were presented by the principal investigator and signed and dated by the subject, the principal investigator, and a witness (Appendix C). A copy of each consent form was placed in each subject's medical records. All medical, dental, and experimental information was completely confidential. Experimental results included only the assigned patient numbers; no names were revealed.

3. Potential Risks. A minimum number of risks were involved in this investigation. One risk was the production of tissue burn due to the use of sodium chloride. Tissue burn, a nonfatal and reversible condition, was of minimal risk due to the small amount of sodium chloride utilized. Further minimization occurred through the subject's employment of written home care instructions which encouraged use of proper proportions of each ingredient. A second risk was that of allergic reaction occurrence. Likelihood of allergic reaction was minimal and could only

have occurred through the presence of unknown sensitization to the medicaments. Occurrence of such a reaction would have prompted immediate performance of emergency medical care protocol. Through instrumentation of scaling and root planing, the third risk of subacute bacterial endocarditis was possible. This risk was minimized by excluding all patients with cardiovascular disorders that may have been predisposed to this condition. If subacute bacterial endocarditis had occurred, medical treatment would have been provided to the subject. If the subject was eligible for medical care as a veteran, the care would be provided by the Veteran's Administration Hospital. If the subject was not eligible for medical care as a veteran, humanitarian care would have been provided. All experimental information remained confidential through use of a coding system for record maintenance. The list of participants' names remained in a locked compartment throughout the study. Termination of the study resulted in immediate destruction of the list.

4. Potential Benefits. The benefits gained through this study included a possible decrease in each of the following: bacterial pathogens associated with periodontal disease, gingival inflammation, and plaque accumulation. These changes would have indicated an increased periodontal health status for the subjects involved. Also, an alternative to traditional periodontal treatment procedures would have been supported through results of this investigation.

This alternative treatment would benefit the involved participants and society in general. During the final appointment, a complete prophylaxis of the remaining quadrants was performed.

5. Risk-Benefit Ratio. The potential benefits of this investigation, which included decreased disease associated microbial quantities, improved periodontal conditions, and alternative periodontal treatment far outweighed the involved risks of tissue burn, allergic reaction, and subacute bacterial endocarditis. Due to careful supervision and periodic monitoring of the subjects, the risks were greatly reduced.

Instrumentation

Methods of measurement to determine the subject sample included periodontal probing, microbial analysis, Gingival Index, and Plaque Index. The periodontal probe is a widely used clinical assessment tool for periodontal disease. Several studies have been performed concerning probe tip location, penetration, controlled probing forces, and/or various types of probes.[69,70,76,105,107] Conflicting results have been observed concerning the validity and reliability of the use of the periodontal probe. One study stated the probe as imprecise in determining pocket depth through measurement of periodontal tissue attachment levels;[76] while another study concluded probe tip level measurements as reflecting a possible degree of connective

tissue reattachment.[105] Despite the conflicting evidence, the periodontal probe continues to be a widely used, important diagnostic instrument by the dental profession.[39]

A total of six areas for each experimental tooth in this investigation were probed and the measurement recorded. There must have existed a minimum of one area on each experimental tooth that ranged in depth from four to seven millimeters. No pocket depth measurement of experimental teeth exceeded seven millimeters.

The Gingival Index is one of several indices utilized for the assessment of particular parameters related to oral health.[1,45,53,64,65,88] The Gingival Index was developed as a system of assessing qualitative changes of the gingival condition.[45] A clear determination was established between the severity and location of the inflammatory lesion. The four areas comprising the circumference of the marginal gingiva (buccal, mesial, distal, and lingual) are involved. Quantitative changes of the periodontium (i.e., periodontal pocket depth, alveolar bone loss) are not considered in evaluation. Inflammatory changes are scored on a scale of 0 to 3; 0 being normal gingiva and 3 being severe inflammation (Appendix I).

The investigation utilized the Gingival Index for the buccal, mesial (mesio-buccal), distal (disto-buccal), and lingual aspects of the experimental teeth (Appendix G). Measurements obtained from any surface may have contained numerical Gingival Index values ranging from 1 to 3.

(The index was revised for purposes of the investigation). Ulceration and/or spontaneous bleeding may not have been present.

The Plaque Index, which distinguished between the severity and location of soft debris aggregates, matched the Gingival Index in purpose and use.[45] Two surfaces involved in the Plaque Index (buccal, lingual) were utilized to evaluate plaque accumulation. Scores of 1 to 3 were obtained for each surface of the experimental teeth in correlation to the criteria demonstrated by the deposit. Criteria for the Plaque Index ranged from a film of plaque to an abundance of soft matter present in the gingival area. (Appendix I) (The Plaque Index also was revised for purposes of the investigation).

Another instrument of measurement utilized in this study was culture analysis. The culture method was used to evaluate anaerobic bacterial components of subgingival plaque samples. Microbial status was investigated to determine the existence of quantitative changes that may have resulted from application of the independent variables in the study. Independent variables included application or non-application of scaling and root planing and the dentifrice of hydrogen peroxide, sodium chloride, sodium bicarbonate versus that of distilled water, sodium bicarbonate.

Explicit differences in microbial content of healthy and diseased periodontal tissue have been indicated to exist

through several investigations concerning samples obtained from the same individual.[4,10,13,18,32,34,40,42,50,58,61,67,74,75,80,82,108] The active state of periodontal disease consists of oral microbiota that differ quantitatively and qualitatively from microbiota associated with periodontal health. Culture medium has been utilized as a tool in the monitoring of the oral microbial status.[35,57,81,85,89] Use of culture medium permits a microbiological approach toward evaluation of sulcular microbiota.[102]

Subgingival microbial sampling was performed every four weeks over an eight-week period. The quantity of the oral microbiota was evaluated and recorded upon a chart containing the microbial form (Appendix F). The bacterial type measured was the anaerobic species Bacteroides melaninogenicus. This microbial characteristic was chosen for measurement because, through numerous investigations, motile gram negative anaerobic micro-organisms have formulated the primary bacterial population of active periodontitis.[4,16,18,32,33,41,42,75,82,84,99]

The initial establishment of the presence of periodontal disease was performed through use of periodontal pocket depth recordings, Gingival Index and Plaque Index recordings, and anaerobic culture media analysis of Bacteroides melaninogenicus. The presence of periodontitis was determined by the principal investigator and confirmed by a licensed dental practitioner through review and evaluation of recorded findings.

All measuring instruments utilized (with exception of the Plaque Index) were standardized prior to initiation of the investigation to determine intrarater reliability. A total of four patients from the Hampton Veteran's Administration Medical Center who met the established criteria of the investigation and who were not study participants were utilized for standardization of the principal investigator. Measurements were performed and recorded first by the principal investigator, then by a licensed dental practitioner. Measurements were compared and evaluated to determine intrarater reliability at the .90 level using the Pearson Product Moment Correlation.

Probing depth was standardized through use of a test/retest method. Pocket measurements were analyzed at six positions for each experimental tooth: disto-buccal beneath the contact area, buccal, mesio-buccal beneath the contact area, disto-lingual beneath the contact area, lingual, and mesio-lingual beneath the contact area. Measurements were recorded from the attached gingiva to the gingival margin by the principal investigator, then re-recorded by a licensed dental practitioner and compared for numerical equivalency. This standardization exercise was performed until the intrarater reliability for pocket readings was determined for all patients at the 0.05 level.

Microbial sampling and evaluation procedures were standardized through repeated supervised procedures. Microbial samplings and quantitative bacterial measurements

were made on each subject. Measurements were performed in designated areas of all four quadrants to ensure establishment of intrarater reliability. Bacterial measurements were supervised by a microbiologist to ensure consistent performance.

The Gingival Index also was standardized through the test/retest procedure. Index measurements were recorded first by the principal investigator, then by a licensed dental practitioner. Evaluation of gingival inflammation was performed upon the buccal and lingual surfaces of all experimental teeth of the four subjects. Intrarater reliability standardization procedures, including probing, microbial sampling and evaluation, and Gingival Index were documented (Appendices M and N).

Statistical Treatment

A two way analysis of variance design was utilized in this investigation. Use of response factor measurements allowed testing for factor difference and interaction in accordance for the three dependent variable measurements. Change scores were calculated from weeks 8 to 0 raw data scores, to enhance reduction of the error variance. The data presented were evaluated in conjunction with the Statistical Analysis System (SAS) software program. The assumptions for all statistical methods utilized encompassed those of a normally distributed population, each population having a common variance. The level of significance was at the 0.05 level.

CHAPTER 4

Results and Discussion

Fifteen subjects were selected from the Hampton Veteran's Administration Medical Center in accordance with a standard criteria. A split mouth experimental design was used; one arch of each patient was randomly assigned to periodontal instrumentation. Half the mouth then was assigned randomly an experimental or a control dentifrice. The experimental dentifrice consisted of hydrogen peroxide, sodium chloride, and sodium bicarbonate, and the control dentifrice consisted of distilled water and sodium bicarbonate. Culture analysis, the Gingival Index, and the Plaque Index were performed initially to provide baseline data and at four and eight weeks. Surfaces chosen for data collection included the deepest pocket depth of two experimental teeth in each quadrant. Evaluation and removal of calculus deposits were performed on one arch in each subject prior to initiation of treatment. Home care procedures involved in the investigation were reviewed at two week intervals. Statistical analyses involved two-way analysis of variance. Change scores were used to enhance reduction of the error variance. The Statistical Analysis System Software program, a computerized statistical package, was used for analysis of data.

Results

Data for hypothesis number one were analyzed to determine if a statistically significant difference existed at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium bicarbonate dentifrice and scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice and scaling and root planing as measured by plated culture media. Simultaneously, data for hypotheses two, three, and four were examined. Data for hypothesis number two were studied to determine if a statistically significant difference existed at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing, and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media. Data for hypothesis number three were examined to determine if a statistically significant difference existed at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by plated culture media. Data for hypothesis number

four were examined to determine if a statistically significant difference existed at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media. Analysis of variance revealed no statistically significant difference at the 0.05 level for either experimental or control groups in the dependent variable measure microbial sampling from baseline to week eight (Table 1). Additionally, no statistically significant difference was found from baseline to week eight between the various treatment combinations used in each respective quadrant. The null hypotheses were not rejected.

Data for hypothesis number five were analyzed to determine if a statistically significant difference existed at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice with scaling and root planing as measured by the Gingival Index. Concurrently, data for hypotheses six, seven, and eight were examined. Data for hypothesis number six were studied to determine if a statistically significant difference existed at the 0.05 level in the gingival condition of patients treated with a hydrogen

Table 1

Analysis of Variance for Microbial Data
for the Four Treatment Methods
from Baseline to Week Eight

Source of Variance	df	ss	ms	F-value	p-value
Model	3	241.7792	80.5931	1.12	0.3487
Error	56	4029.2667	71.9512		
Total	59	4271.0458			
Home Care Regimen	1	121.8375		1.69	0.1985
Instrumentation	1	119.0042		1.65	0.2037
Interaction	1	0.9375		0.01	0.9095

$r^2 = 0.056609$

peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index. Data for hypothesis number seven were studied to determine if a statistically significant difference existed at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Gingival Index. Data for hypothesis number eight were examined to determine if a statistically significant difference existed at the 0.05 level in the gingival condition of patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index. Analysis of variance disclosed no significant difference at the 0.05 level for either experimental or control group in the dependent variable measure gingival inflammation from baseline to week eight (Table 2). Concurrently, no statistically significant difference was revealed from baseline to week eight between the various treatment combinations used in each respective quadrant. The null hypotheses were not rejected.

Table 2

Analysis of Variance for Gingival Index Data
for the Four Treatment Methods
from Baseline to Week Eight

Source of Variance	df	ss	ms	F-value	p-value
Model	3	0.3052	0.1.17	0.64	0.5915
Error	56	8.8812	0.1586		
Total	59	9.1864			
Home Care Regimen	1	0.2667		1.68	0.2001
Instrumentation	1	0.0010		0.01	0.9357
Interaction	1	0.0375		0.24	0.6287

$r^2 = 0.033224$

Data for hypothesis number nine were analyzed to determine if a statistically significant difference existed at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice with scaling and root planing as measured by the Plaque Index. Simultaneously, data for hypotheses ten, eleven, and twelve were evaluated. Data for hypothesis number ten were studied to determine if a statistically significant difference existed at tthe 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index. Data for hypothesis number eleven were examined to determine if a statistically significant difference existed at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Plaque Index. Data for hypothesis number twelve were studied to determine if a statistically significant difference existed at the 0.05 level in the plaque accumulation of patients treated with a

sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index. Analysis of variance revealed no statistically significant difference at the 0.05 level for either experimental or control group in the dependent variable measure, plaque accumulation, from baseline to week eight (Table 3). Also revealed was no significant difference from baseline to week eight between the various treatment combinations used in each respective quadrant. The null hypotheses were not rejected.

Discussion

Analysis of variance for microbial data revealed no statistically significant difference between utilization of the home care regimens upon the quantitative composition of Bacteroides melaninogenicus. Neither dentifrice was shown to provide additional benefits over the opposing dentifrice. These findings are consistent with results obtained by West and King [110] and Cerra and Killoy[9] in which both compared a hydrogen peroxide paste to conventional toothpaste or tooth powder.

Results obtained when the additional variable of scaling and root planing was combined with each of the two dentifrices also revealed no significant differences. Comparison of arches receiving scaling and root planing revealed no statistically significant difference. These results are not consistent with those achieved by Tagge

Table 3

Analysis of Variance for Plaque Index Data
for the Four Treatment Methods
from Baseline to Week Eight

Source of Variance	df	ss	ms	F-value	p-value
Model	3	0.4615	0.1538	0.99	0.4029
Error	56	8.6750	0.1549		
Total	59	9.1365			
Home Care Regimen	1	0.3760		2.43	0.1249
Instrumentation	1	0.0010		0.01	0.9349
Interaction	1	0.0844		0.54	0.4636

$r^2 = 0.050507$

et al.[97] Tagge and his associates concluded that root planing and oral hygiene produced improved microscopic scores as compared to oral hygiene alone. Unexpected results achieved in the present investigation may have been due to the dedicated use of each dentifrice by the subjects which would maintain bacterial colonies associated with plaque and periodontal disease at a minimum level. The result also may have been due to the minimal presence of calcified deposits in several subjects. Removal of these deposits may have produced insignificant inflammatory changes because of the small influence of the deposits upon the gingival inflammatory state. Marginal gingival inflammation, as measured by the Gingival Index, may have been minimally affected by the presence or absence of the subgingival calcified deposits.

Gingival Index values revealed no statistically significant difference as measured by analysis of variance between the use of the two home care regimens. Neither dentifrice was shown to provide additional benefits over the other. There exists a consistency in these results and those obtained by Wolff et al.[112] who compared topical antimicrobial use of hydrogen peroxide and sodium chloride with conventional home therapy of dental floss and nonfluoridated toothpaste.

Addition of the scaling and root planing variable to each of the two dentifrice regimens yielded identical results of no statistically significant difference. No

significant difference was present between arches receiving scaling and root planing and those receiving no scaling and root planing. Good plaque control or the presence of little calculus prior to instrumentation may provide some reason for the lack of significant difference observed. Tagge et al.[97] found conflicting results in comparison to the results achieved in this investigation. Greater plaque accumulation was present following eight weeks of treatment on surfaces that did not receive root planing instrumentation and were greatly roughened in nature. Tabita et al.[96] presented results inconsistent with this investigation. Fourteen days after scaling, root planing, and polishing, subgingival bacterial plaque had reformed, and no significant difference between gingival scores was found. Smulow et al.[89] presented results which paralleled those achieved in this investigation. A reduction in Gingival and Plaque Indices throughout treatment was seen most consistently around teeth that were polished daily with or without initial subgingival scaling. Daily polishing of the tooth surfaces resulted in the minimal presence of plaque deposits.

Analysis of variance for Plaque Index values revealed no statistically significant difference between use of the two home care regimens. Neither was shown to provide additional benefits over the other. Wolff et al.[112] achieved similar results to those in this investigation. The similar abrasive qualities of both experimental and control

dentifrices could provide a rational reason for the similar achievements of plaque control. The conscientious oral hygiene procedures shown by the subjects also played an important role in the ultimate decrease of Plaque Index values. Results were identical when the additional variable of scaling and root planing was combined with each home care regimen.

Results demonstrated that no statistically significant differences existed between the two dentifrices used in the home care regimens. The addition of scaling and root planing did not provide additional significant benefits.

An influence was produced by the factor of time when combined with a specific home care regimen in this investigation, thus yielding a positive amount of clinical significance. Use of either dentifrice as part of the home care regimen involved in this investigation produced improved periodontal health over the eight week time period. Therefore, dedicated performance by an individual of an established oral hygiene routine may provide maintenance or improvement of their periodontal status.

Limitations of the study should be discussed when interpreting results. The utilization of the single microbial species, Bacteroides melaninogenicus, may have presented a limitation. Through the addition of other microbiota, not only would further information be produced, but also the positive results achieved with B. melaninogenicus may be supported.

The addition of other clinical parameters also may have presented extra beneficial results. Parameters such as clinical attachment levels and periodontal probing would allow pocket depth measurement changes, and thus, any benefits achieved, to be recorded.

Additionally, inclusion of subjects from various backgrounds as opposed to the intact group used in this investigation would allow generalization of results to other populations.

CHAPTER 5

Summary and Conclusions

The dental profession is one which is responsible for maintenance of the dentition and oral cavity in an optimum state of health. Periodontal disease poses a threat to this healthy condition and if allowed to continue, may result in complete destruction of the periodontium. Various treatments available for periodontal disease currently are being utilized; however, no single treatment may be said to possess significant advantage over another.

Fifteen subjects were selected from the Hampton Veteran's Administration Medical Center in accordance with a standard criteria. The split mouth experimental design was used. Prior to initiation of treatment, one arch of each subject was randomly assigned to evaluation and removal of calculus deposits through periodontal instrumentation. Half of the mouth (consisting of one maxillary quadrant and its opposing mandibular quadrant) then was assigned randomly an experimental or control dentifrice. Dentifrices consisted of hydrogen peroxide, sodium chloride, sodium bicarbonate, and distilled water, sodium bicarbonate, respectively. Culture analysis, the Gingival Index, and the Plaque Index were performed initially to provide baseline data and at four and eight weeks by the principal investigator.

Throughout the investigation, the sides of the mouth and their corresponding assigned dentifrice were unknown to the principal investigator, thus constituting a blind research approach. Statistical analyses performed involved a two-way analysis of variance. The Statistical Analysis System Software program, a computerized statistical package, was used for analysis of data.

Findings from statistical analyses revealed no statistically significant differences at the 0.05 level for the experimental variable of home care regimens, therefore none of the following tested hypotheses were rejected:

(H_{0_1}) There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides. melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice and scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice and scaling and root planing as measured by plated culture media.

(H_{0_2}) There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides. melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media.

(H_{0_5}) There is no statistically significant difference

at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice with scaling and root planing as measured by the Gingival Index.

(H_{0_6}) There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index.

(H_{0_9}) There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice with scaling and root planing as measured by the Plaque Index.

($H_{0_{10}}$) There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index.

Findings from statistical analyses also revealed no statistically significant interaction at the 0.05 level between the type of home care regimen and the implementation of scaling and root planing, therefore none of the following tested hypotheses were rejected:

(H_{0_3}) There is no statistically significant difference at the 0.05 level in the microbial count of Bacteroides melaninogenicus in patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by plated culture media.

(H_{0_4}) There is no statistically significant difference at the 0.05 level in the bicrobial count of Bacteroides melaninogenicus in patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by plated culture media.

(H_{0_7}) There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Gingival Index.

(H_{0_8}) There is no statistically significant difference at the 0.05 level in the gingival condition of patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Gingival Index.

($H_{0_{11}}$) There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice with scaling and root planing and in patients treated with the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice without scaling and root planing as measured by the Plaque Index.

($H_{0_{12}}$) There is no statistically significant difference at the 0.05 level in the plaque accumulation of patients treated with a sodium bicarbonate, distilled water dentifrice with scaling and root planing and in patients treated with the sodium bicarbonate, distilled water dentifrice without scaling and root planing as measured by the Plaque Index.

Considering the discussion and limitations of the study, the following conclusions are offered:

1. The hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice and the sodium bicarbonate, distilled water dentifrice are effective agents in improving periodontal health through their effective reduction of B.

melaninogenicus colonies, gingival inflammation, and plaque accumulation in patients with mild to moderate periodontitis.

2. The experimental dentifrice does not provide greater beneficial value than the control dentifrice in achieving periodontal health.

3. Scaling and root planing instrumentation clinically, but not statistically, supplements a thorough home care regimen using either the experimental or the control dentifrice in achieving periodontal health.

Considering the results and overall design of this investigation, the following recommendations are offered:

1. This investigation should be replicated for verification of the achieved results.

2. This investigation should be repeated on a long-term basis to observe the associated benefits of the hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice.

3. Comparison of the experimental dentifrice with a control dentifrice composed solely of distilled water may yield results favoring one dentifrice over the other.

4. Replication of the investigation utilizing a larger sample size is recommended to substantiate results.

5. The investigation should be repeated utilizing additional microbial parameters to provide additional and supportive results.

6. The investigation should be repeated utilizing

additional clinical parameters, such as periodontal probing or clinical attachment levels, to provide additional and supportive information.

7. Replication of the investigation utilizing subjects with moderate to severe degrees of periodontitis is recommended to provide information concerning associated benefits through use of the experimental dentifrice home care regimen.

8. Long-term research is needed to determine effectiveness of the experimental dentifrice on moderate to severe periodontitis in surgically and non-surgically treated patients.

This investigation revealed that the dentifrice of hydrogen peroxide, sodium chloride, sodium bicarbonate is effective when applied as part of a daily homecare regimen in improving periodontal health conditions. The dentifrice was also found to be of beneficial value for maintaining basic periodontal health when used as part of a daily regimen. The hydrogen peroxide, sodium chloride, sodium bicarbonate dentifrice was not shown to provide additional benefits in comparison to a sodium bicarbonate, distilled water dentifrice. Among the various treatments available for periodontal disease, there currently remains no single treatment possessing significant advantage over others.

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RESEARCH PARADIGM

	H_2O_2 NaCl $NaHCO_3$	DISTILLED H_2O $NaHCO_3$
SCALING AND ROOT PLANING	EXPERIMENTAL DENTIFRICE + SCALING AND ROOT PLANING	CONTROL DENTIFRICE + SCALING AND ROOT PLANING
NO SCALING AND ROOT PLANING	EXPERIMENTAL DENTIFRICE + NO SCALING AND ROOT PLANING	CONTROL DENTIFRICE + NO SCALING AND ROOT PLANING

APPENDIX B

QUADRANT AND ARCH RANDOMIZATION PARADIGM

QUADRANT AND ARCH RANDOMIZATION PARADIGM *

Patient Number	Experimental Side	Control Side	Scale & Root Plane Maxillary	Mandibular
1				
2				
3				
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*Designed Specifically for This Investigation

SUBJECT CONSENT FORM

Project Name: Effectiveness of a Hydrogen Peroxide, Sodium Chloride, Sodium Bicarbonate Dentifrice Upon Microbiota Associated with Periodontitis.

Investigator: Jill M. Modi, R.D.H., B.S.

Date: _____

You are invited to participate in a study that will be held at the Hampton Veteran's Administration Medical Center dental clinic. The study will involve prescribed dentifrices consisting of distilled water, sodium bicarbonate (baking sode) and sodium chloride (table salt), or distilled water and sodium bicarbonate (baking soda). The teeth in one half of your mouth will be cleaned. This will allow the effects of your home care regimen and the cleaning of your teeth, to be compared. We hope to learn if any significant relationship exists between the use of hydrogen perioxide, sodium bicarbonate, and sodium chloride as a routine toothpaste and pyhorrea activity. The discovery of this relationship relies mainly upon your self-discipline and dedicated daily performance of the prescribed home care regimen. We are unable to guarantee that any benefits will be received through participation in this study.

Please read the following description carefully to ensure complete understanding of the involved scientific investigation prior to signing this document.

This is to certify that I, _____, hereby agree to participate as a volunteer in a scientific investigation as part of the educational and research program at Old Dominion University, in conjunction with the Hampton Veteran's Administration Medical Center dental clinic, under the supervision of Jill M. Modi, R.D.H., B.S. (Principal Investigator).

I understand that I will be one of twenty patients involved in this study, and that the location of the study will be the Hampton Veteran's Administration Medical Center dental clinic. I have been chosen to participate in this investigation because I currently have a pyhorrea condition and do not have any medical complications that would affect my participation. I understand that a total of five appointments will be involved, including a two and

one half hour first appointment, followed by consequent appointments of one hour maximum time limits. A total of eight weeks will be utilized, with each appointment occurring at two-week intervals. I understand that any results of this study will rely upon my faithful daily appearance at every appointment scheduled. I will complete a medical history form and verify that all involved questions have been answered truthfully to the best of my knowledge and ability. Following the investigation, my oral hygiene treatment will be continued and my prophylaxis (tooth cleaning) will be completed at the Hampton Veteran's Administration Medical Center Dental Clinic.

I understand that there is one possible risk involved in this investigation. The risk, which is completely reversible, is that of tissue burn (from use of the table salt). I have been informed that the occurrence of this risk is slight in possibility due to the conditions of the study. There are also several possible benefits: a decrease in the presence of germs associated with pyhorrea, a decrease in the amount of plaque present, and a related increase in the health of my gums. I also understand that I will be using both experimental and control toothpastes. We do not guarantee or promise that you will receive any benefits from this study.

The investigation and the nature of my participation have been described and explained to me, and I understand the explanation. I understand that I am one of twenty individuals participating in this research project. I further understand that I may withdraw from the project at any time, without penalty or prejudice toward or from the Hampton Veteran's Administration Medical Center Dental Clinic or Old Dominion University.

I have been afforded an opportunity to ask questions concerning the purpose of this project and all such questions have been answered to my satisfaction. I understand that should I have additional questions in the future about this project or the manner in which it is conducted, I may contact Jill M. Modi (Principal Investigator) at (804) 622-2510, or Dr. Norman Glasscock (Research Supervision) at (804) 722-9961.

I understand that participation in the investigation is strictly voluntary and no monetary compensation will be received. I have been informed that results of this study may be published or orally dictated, but I will not be individually identified. No information identifying me will be released to persons outside the research team without the team first obtaining my written permission. I acknowledge that I have been advised of how I may obtain a copy of the results of this research project, and that upon my making such a request, a copy will be provided without charge.

I acknowledge that I was informed about any possible risks to my health and well being that may be associated with my participation in this research. I understand that no medical or psychological assistance will be made available to me by either Old Dominion University or any member of the research team as a result of any physical or emotional harm I may experience as a result of this research project.

In the event I sustain physical injury as a result of participation in this investigation, if I am eligible for medical care as a veteran, all necessary and appropriate care will be provided. If I am not eligible for medical care as a veteran, humanitarian emergency care will nevertheless be provided. I understand no medical or psychological assistance will be made available to me by Old Dominion University.

I have been informed that I have the right to contact the Old Dominion University Institutional Review Board for the Protection of Human Subjects or the Hampton Veteran's Administration Medical Center Committee for the Protection of Human Subjects should I wish to express any opinions regarding the conduct of this study. I further understand that all or a portion of the records concerning this study may be reviewed by the U.S. Food and Drug Administration.

Having read the information provided in this document, I hereby consent to be a participant in this investigation. My signature indicates my agreement to participate and to perform all necessary procedures to the best of my ability.

_____ _____

Signature of Volunteer Date

_____ _____

Witnessed by Date

_____ _____

Signature of Investigator Date

_____ _____

Witnessed by Date

APPENDIX D

INITIAL PATIENT INFORMATION INSTRUCTIONAL TECHNIQUE

INITIAL PATIENT INFORMATION INSTRUCTIONAL TECHNIQUE

Preventive Therapist's Dialogue

"Good morning (or good afternoon), _____."

"My name is preventative therapist's or principal investigator's name."

"The study you may be chosen to participate in will involve a total of 20 people. You may be selected as one of these 20 because you have certain characteristics that are needed for this project. The criteria you need to meet include: a periodontally diseased mouth, which will be explained later; age 20 to 70; at least twenty natural teeth, including eight designated experimental teeth; and pocket depths between your teeth and gums no deeper than four to seven millimeters on those eight teeth. You must also be able to perform your own oral hygiene, like brushing and flossing, at an excellent level. Also, you must not be presently taking any medications that may interfere with the health condition of your gums."

"The study will last a total of eight weeks."

"You will be given specific home care treatments to follow every morning and night. They must be followed faithfully every day for the research to work, so each of you will play an indispensible role in the success of this project! You will be using a toothpaste made of hydrogen peroxide, distilled water, baking soda, and table salt; and a paste of distilled water and baking soda. At the first appointment, four samples of plaque will be taken from your mouth so they can be analyzed with culture media to tell what kinds of germs are present. We're also going to see how inflamed your gums are and how much plaque you have in your mouth. After this, you will be given an instruction sheet that tells you how you will be cleaning your teeth and gums for the next eight weeks. You will also be taught the technique, step-by-step. Next, one arch of your mouth will be cleaned. The other arch is left alone so we can see the effects of your assigned toothpastes on this side as compared to the other side."

"The next time we see you will be two weeks later. At that time, you will review the home care techniques with the therapist to make sure you are performing the procedures in the way you were first taught. You will do this every two weeks."

"Two weeks after this second appointment we will see you to take four more plaque samples to find out if the germs have changed in number. We will also record any inflammation present."

"After this third appointment, we will see you every two weeks for two more appointments (this will equal about eight weeks). All of them are very important to the overall success of the study. At the end of the study, we will calculate all the data to see what effects each toothpaste had on the teeth that were cleaned and on the teeth that were not cleaned. These effects will be noted by the number of germs, amount of inflammation, and amount of plaque present in each section of your mouth."

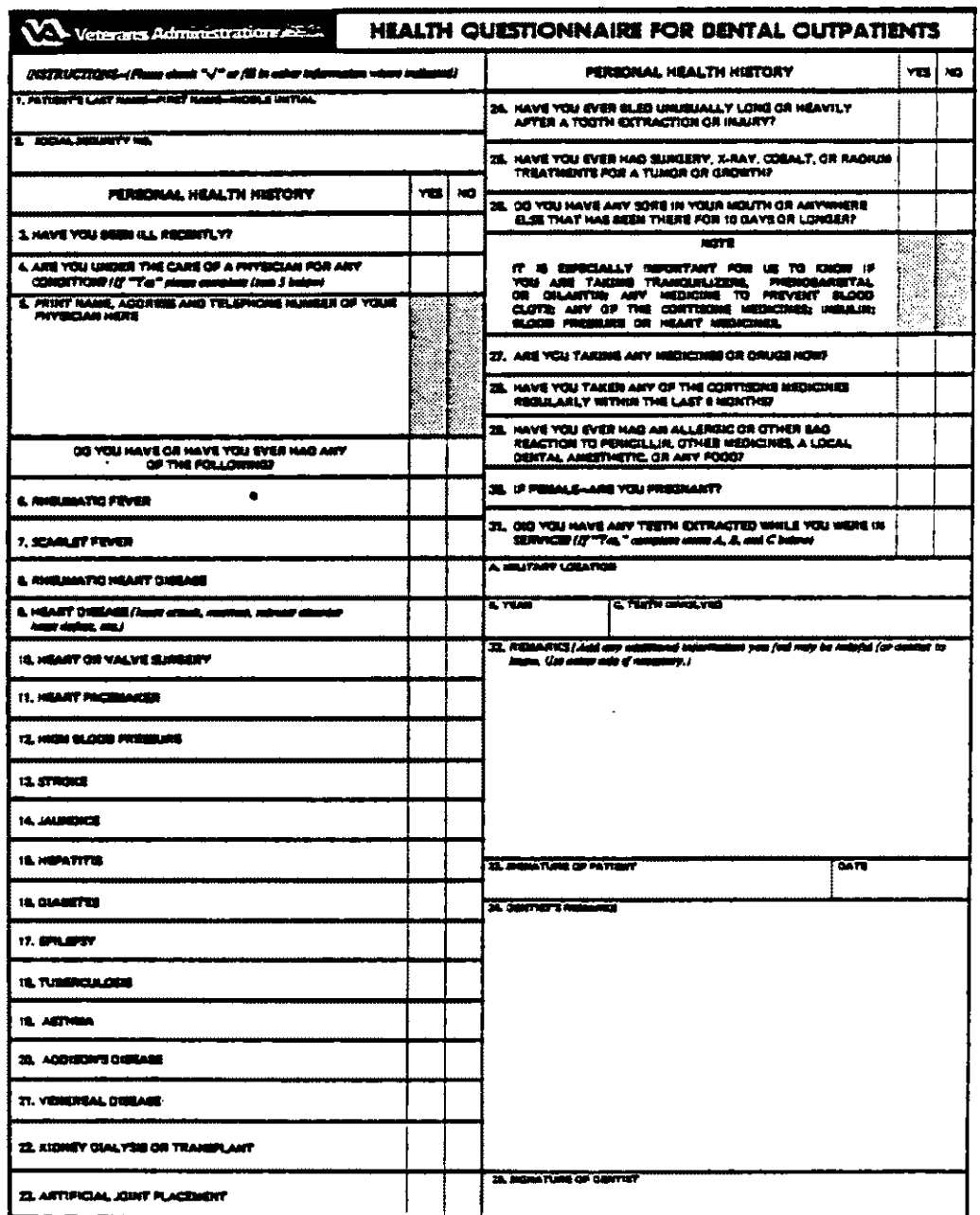
"After the study, each of you will have continued dental treatment through the dental clinic here at the Hampton Veteran's Administration Medical Center."

"There are a few risks that may be present with this study. The use of salt on your gums may cause a slight burn which will be detected by redness and a burning sensation. This is reversible and in no way harmful to your health. Also, this tissue burn possibility will be minimized by your using the exact amount of salt prescribed and by following your written instruction sheet. A second risk is that of an allergic reaction, which is minimal and will occur only if you have an unknown allergy to the ingredients. If a reaction occurs, immediate performance of emergency medical care will take place. The cleaning of your teeth also carries a minimum potential risk from possible bacterial infection, but this is present with any professional cleaning treatment. Emergency personnel and equipment will be readily available if needed. The possible benefits of the study include a decrease in the presence of germs associated with periodontal disease, a decrease in the amount of inflammation and plaque in your mouth, and a related increase in periodontal health. Since you will be carefully monitored and supervised, the risks are not considered a restriction in this study."

"Thank you very much for helping us in this research. If chosen, your oral hygiene actions over the next eight weeks will have the power of making the study a success through faithful, everyday performance, or a failure. We will hopefully see an improvement in your gum condition through use of this technique."

"Thank you very much for your time and attention."

MEDICAL HISTORY FORM



Veterans Administration

HEALTH QUESTIONNAIRE FOR DENTAL OUTPATIENTS

(INSTRUCTIONS—(Please check "√" or fill in other information where indicated)

1. PATIENT'S LAST NAME—FIRST NAME—MIDDLE INITIAL

2. SOCIAL SECURITY NO.

PERSONAL HEALTH HISTORY	YES	NO
3. HAVE YOU BEEN ILL RECENTLY?		
4. ARE YOU UNDER THE CARE OF A PHYSICIAN FOR ANY CONDITION? *(If "Yes" please complete item 5 below)*		
5. PRINT NAME, ADDRESS AND TELEPHONE NUMBER OF YOUR PHYSICIAN HERE		
DO YOU HAVE OR HAVE YOU EVER HAD ANY OF THE FOLLOWING?		
6. RHEUMATIC FEVER		
7. SCARLET FEVER		
8. RHEUMATIC HEART DISEASE		
9. HEART DISEASE *(heart attack, murmur, mitral valve/aortic heart defect, etc.)*		
10. HEART OR VALVE SURGERY		
11. HEART PACEMAKER		
12. HIGH BLOOD PRESSURE		
13. STROKE		
14. JAUNDICE		
15. HEPATITIS		
16. DIABETES		
17. EPILEPSY		
18. TUBERCULOSIS		
19. ASTHMA		
20. ADDISON'S DISEASE		
21. VENEREAL DISEASE		
22. KIDNEY DIALYSIS OR TRANSPLANT		
23. ARTIFICIAL JOINT PLACEMENT		

PERSONAL HEALTH HISTORY	YES	NO
24. HAVE YOU EVER BLED UNUSUALLY LONG OR HEAVILY AFTER A TOOTH EXTRACTION OR INJURY?		
25. HAVE YOU EVER HAD SURGERY, X-RAY, COBALT, OR RADIUM TREATMENTS FOR A TUMOR OR GROWTH?		
26. DO YOU HAVE ANY SORE IN YOUR MOUTH OR ANYWHERE ELSE THAT HAS BEEN THERE FOR 10 DAYS OR LONGER?		
NOTE IT IS ESPECIALLY IMPORTANT FOR US TO KNOW IF YOU ARE TAKING TRANQUILIZERS, PHENOBARBITAL OR ORLANTIN; ANY MEDICINE TO PREVENT BLOOD CLOTS; ANY OF THE CORTISONE MEDICINES; INSULIN; BLOOD PRESSURE OR HEART MEDICINES.		
27. ARE YOU TAKING ANY MEDICINES OR DRUGS NOW?		
28. HAVE YOU TAKEN ANY OF THE CORTISONE MEDICINES REGULARLY WITHIN THE LAST 6 MONTHS?		
29. HAVE YOU EVER HAD AN ALLERGIC OR OTHER BAD REACTION TO PENICILLIN, OTHER MEDICINES, A LOCAL DENTAL ANESTHETIC, OR ANY FOOD?		
30. IF FEMALE—ARE YOU PREGNANT?		
31. DID YOU HAVE ANY TEETH EXTRACTED WHILE YOU WERE IN SERVICE? *(If "Yes," complete items A, B, and C below)*		

A. MILITARY LOCATION

B. YEAR | C. TEETH EXTRACTED

32. REMARKS *(Add any additional information you feel may be helpful or needed to know. Use reverse side if necessary.)*

33. SIGNATURE OF PATIENT | DATE

34. DENTIST'S REMARKS

35. SIGNATURE OF DENTIST

VA FORM JAN 1958 **10-2570a**

EXISTING STOCK OF VA FORM 10-2570a, AUG 1970, WILL BE USED.

* U.S. GOVERNMENT PRINTING OFFICE: 1958 — 461-466/3016

APPENDIX F

LÖE´S GINGIVAL INDEX CHART CRITERIA

LÖE'S GINGIVAL INDEX CHART CRITERIA

Information:

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

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

3 = Severe inflammation--redness, oedema, and Ulceration. Tendency to spontaneous bleeding.

Experimental teeth:

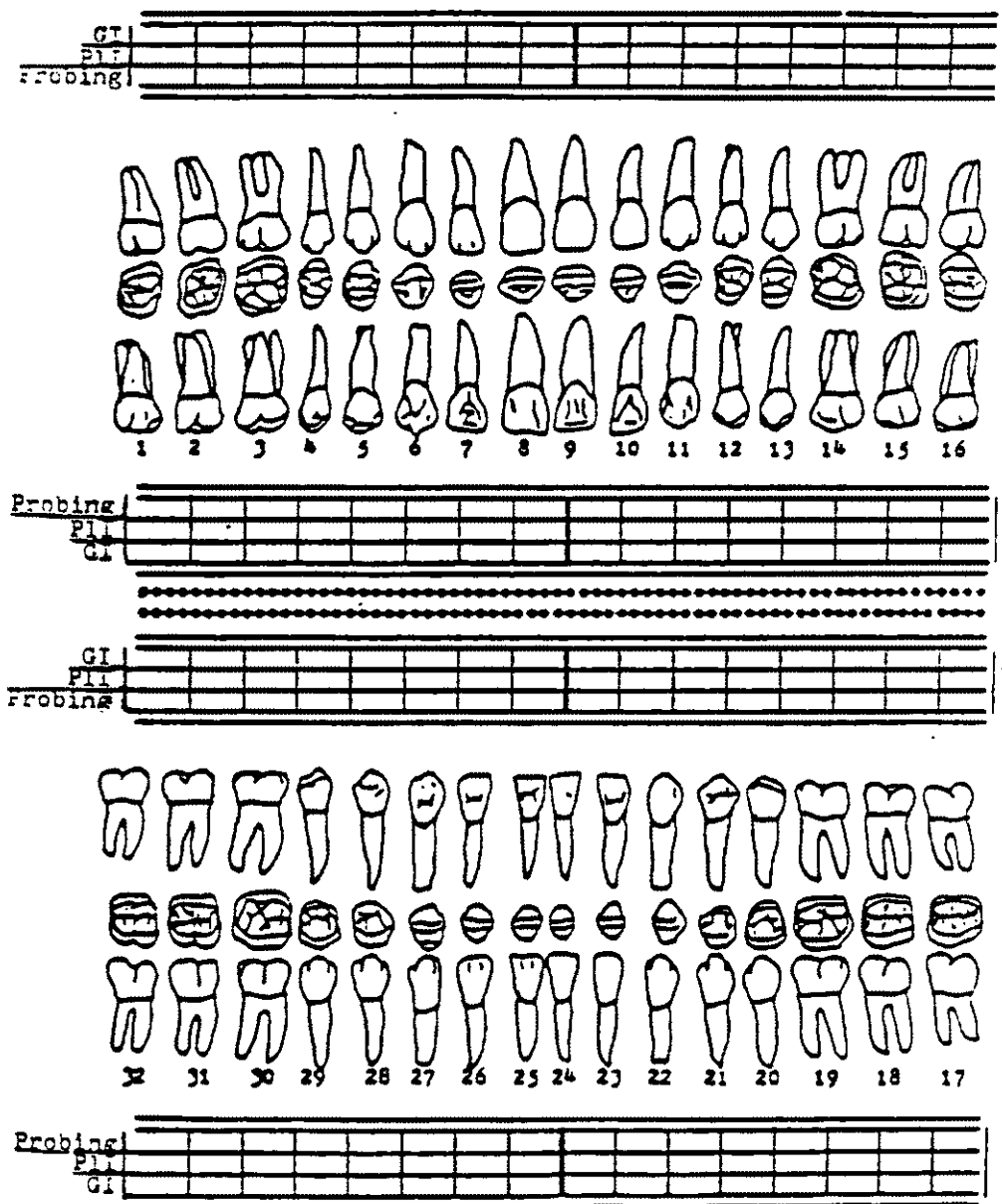
First molar and second premolar in each quadrant. If one or both of these teeth is/are absent, the first premolar and/or second molar was/were included, respectively.

APPENDIX G

PERIODONTAL PROBING, PLAQUE INDEX, AND GINGIVAL INDEX CHART

PERIODONTAL PROBING, PLAQUE INDEX, AND GINGIVAL INDEX CHART

PATIENT'S NAME _____ DATE _____



APPENDIX H

LÖE´S PLAQUE INDEX CHART CRITERIA

LÖE'S PLAQUE INDEX CHART CRITERIA

Information:

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.

Experimental Teeth:

First molar and second premolar in each quadrant.
If one or both of these teeth is/are absent, the first premolar and/or second molar was/were included, respectively.

APPENDIX I

MICROBIAL EVALUATION CHART

MICROBIAL EVALUATION CHART *

Information:

Experimental Tooth Number:

3, 4 (5 or 2, respectively, if either is absent)
13, 14 (12 or 15, respectively, if either is absent)
19, 20 (21 or 18, respectively, if either is absent)
29, 30 (28 or 31, respectively, if either is absent)

Tooth Surface:

DB = Disto-buccal
B = Buccal
MB = Mesio-buccal
DL = Disto-lingual
L = Lingual
ML = Mesio-lingual

Tooth Utilized:

Deepest periodontal pocket initially detected among the experimental teeth in each quadrant.

Patient Number _____

Patient Information

Appointment Number	Tooth Number	*Bacteroides melaninogenicus* Number of Colonies
1		
2		
3		

*Designed Specifically for This Investigation

APPENDIX J

PATIENT HOME CARE INSTRUCTIONAL TECHNIQUE

PATIENT HOME CARE INSTRUCTIONAL TECHNIQUE

Preventive Therapist's Dialogue

"Good morning (or good afternoon), patient's name."

"My name is preventive therapist's name."

"As you know, you have been selected for this study because you have a gum disease known as periodontitis. This is an inflammatory condition caused by certain types of bacteria (germs) that are found in your mouth. These bacteria are collectively known as plaque. Plaque is a sticky invisible coating or film which collects on your teeth every day and must be removed mechanically through daily brushing and flossing. If not removed, the bacteria form products that irritate your gums. This causes your gums to become red and inflamed as your body fights to combat these invaders. If allowed to continue, a space will be formed between your teeth and gums that will allow more plaque to accumulate. If the plaque is not removed within 24 hours of its formation, it will harden into calculus (tartar). Once this calcification occurs a rough niche is formed that serves as a place for the formation of more plaque. Calculus can only be removed professionally by your dentist or dental hygienist. If the supporting structures of your teeth and gums become involved in the infection, deep "pockets" will form around the teeth and cause eventual tooth mobility and tooth loss. This infectious process is the main cause of tooth loss in the adult population of the United States."

"However, these consequences of periodontal disease can be prevented. A good daily oral hygiene routine that includes brushing and flossing; along with routine professional dental visits is the key to this prevention. Home care procedures must be performed every day to be effective."

"Now I will show you the methods you will use during the next eight weeks for this study for the daily removal of plaque from your teeth. You will be given (1) a toothbrush, dental floss, rubber tip stimulator, 3% hydrogen peroxide, distilled water, baking soda, and table salt (for experimental side); and (2) a toothbrush, dental floss, rubber tip stimulator, distilled water, and baking soda (for control side). First you will clean the control side of your mouth. You will place on teaspoon distilled water in a bowl, and moisten the bristles of the toothbrush labeled "water." Next, add four teaspoons baking soda and mix to form a paste. Divide the control side into two parts (upper and lower)."

"Starting with the upper part, you will perform the following steps. Each time you do this, begin with the

back tooth and continue forward to the central midline. First clean the outside surfaces, then the inside. (This will prevent the paste from diluting before you reach the inside surfaces.) To begin, pick up some paste with the toothbrush bristles. Next, smear it along the gumline for all the teeth in part 1 (eigher the outside OR the inside surfaces), beginning in the back and continuing to the midline. Now you will use the rubber tip stimulator to help distribute the paste into the space between the tooth and the gums (which is also known as the sulcus). To use the rubber tip stimulator, you will place the tip at a 45° angle gently below the gumline. Now, slowly vibrate the tip around each tooth, making sure that the tip remains against the tooth, NOT against the gum. Do NOT rinse your mouth yet."

"The next step is brushing with your toothbrush. This is a sulcus toothbrush, which will allow you to perform this technique better than if you were to use a regular toothbrush. Place the toothbrush bristles at a 45° angle toward the gumline gently into the sulcus. Now, vibrate the bristles back and forth with short strokes; approximately ten total strokes for each area of the teeth. Immediately after you vibrate the bristles, roll them toward the chewing surface of your tooth (away from the gumline). Continue this until the midline is reached. You still do NOT rinse."

"Now smear the paste on the inside surfaces and again use the rubber tip stimulator and sulcus toothbrush."

"Brush the chewing surfaces of your teeth with back and forth strokes."

"Your next step is to floss with the unwaxed floss we've given you. Break off a 12-to 15-inch length of floss, and wrap one end around the middle finger of your right hand, and the other around the middle finger of your left hand. Do this until approximately three to four inches remain. Use your thumbs and index fingers as guides to control the flossing motion. Now place the floss behind the last tooth and make a "c" shape by bringing the ends forward. Using an up and down scraping motion, floss the back of the last tooth from the top of the tooth to the possible depth of the sulcus beneath the gum. You will know when you've reached the stoping point on your downward motion to the sulcus when you meet slight resistance. When you feel this resistance, do NOT try to force the floss down, but reverse directions and begin your upward stoke. Use a total of ten up and down strokes on each surface. Next, move to the front side of the last tooth. To floss this, gently "see-saw" the floss between the two teeth until the floss is capable of free up and down movement. Now floss the front side of the tooth. You also floss the back side of the

next tooth without removing the floss completely by bringing it up and over the papilla (tissue wedge) that is in-between the teeth. The floss will now rest upon the next tooth. Continue this for all teeth until the midline is reached. You still do NOT rinse!"

"Continue this sequence of smearing the paste, using the rubber tip stimulator, brushing, and flossing for the lower part. Only after both parts are completed may you rinse with water. At that point, you will rinse well with several mouthfuls of water."

"Now for the other side of your mouth, do the exact same steps, substituting hydrogen peroxide and distilled water for the distilled water, and baking soda and salt for the baking soda."

"This technique will remove the bacterial plaque as well as distribute the paste into the sulcus. By getting the paste into the sulcus, we can eventually see what effects it has upon the bacteria present in the sulcus."

(*This explanation will first be given verbally by the preventive therapist along with a step-by-step demonstration. The demonstration will first be upon the dentoform (using a designated set of home care implements), then in the patient's mouth (using his own home care implements). Next, the patient will demonstrate, step-by-step, first on the dentoform, then in his own mouth.*)

"At each appointment we will review your performance of this home care method. At every other appointment we will take a total of four samples of plaque from different areas of your mouth so that we can see how many germs are present. The different areas used for the samples will be determined by the pocket depth measurements we took earlier. One area from every quadrant will be selected according to the deepest pocket measurement recorded. We will also determine how inflamed your gums are and how much plaque is present in the four specified areas of your mouth."

"Remember that the success, or failure, of this research depends on you and your faithful use of this home care technique. We will hopefully see an improvement in your gum condition if you use this technique as you've been instructed. If you have any questions at all, please feel free to call me at 440-4310. Thank you very much for your attention, and I know you'll do a fine job with the technique. Good Luck!"

(*At appointments two through five, the home care regimen will be reviewed by the preventive therapist.*)

"Good morning or good afternoon, patient's name."

"Today we're going to review your home care method. Please show me the steps you have been using and how you have been cleaning your teeth since the last time we met." (Patient demonstrates entire technique upon his own mouth. Necessary corrections are made by the preventive therapist.)

"I hope today has added to your confidence in using the home care technique. Thank you for your cooperation, and keep up the good work!"

APPENDIX K

PATIENT HOME CARE INSTRUCTION GUIDE

PATIENT HOME CARE INSTRUCTION GUIDE

Part I.

On the _____ side:

A. 1. Place 1 teaspoon distilled water in bowl.
2. Moisten bristles of toothbrush labeled "water."
3. Add 3 1/2 teaspoon baking soda
(A paste will be formed.)

B. Divide this side of your mouth into 2 parts (upper and lower)

First perform these steps (completely) on the upper part, then repeat for the lower part. Each time begin with the back tooth and continue forward to the front central midline. First clean the outside surfaces, then the inside.

1. Pick up some paste with the toothbrush bristles.
2. Smear it along the gumline for all the teeth in Part I.
3. Use the rubber tip stimulator as instructed to help distribute the paste.
4. Brush with the sulcus toothbrush.
5. Floss. (outside and inside)
6. Do NOT rinse. Continue to the bottom half.

C. Rinse thoroughly before going on to Part II.

Part II.

On the _____ side:

A. 1. Place 1/2 teaspoon hydrogen peroxide and 1/2 teaspoon distilled water in bowl.
2. Mosten bristles of toothbrush labeled "peroxide".
3. Add 1/4 teaspoon salt and 3 1/2 teaspoon baking soda (A paste will be formed.)

B. Divide this side of your mouth into 2 parts (upper and lower).

First perform these steps (completely) on the upper part. Then repeat for the lower part. Each time begin with the back tooth and continue forward to the front central midline. First clean the outside surfaces, then the inside.

1. Pick up some paste with the toothbrush bristles.

2. Smear it along the gumline for all the teeth in Part I.
3. Use the rubber tip stimulator as instructed to help distribute the paste.
4. Brush with the sulcus toothbrush.
5. Floss. (outside and inside)
6. Do NOT rinse. Continue to the bottom half.

C. Rinse thoroughly.

APPENDIX L

CRITERIA FOR PATIENT SELECTION

CRITERIA FOR PATIENT SELECTION

Goal: 30 patients
Currently registered at Hampton
Veteran's Administration Medical Center
Male or Female

1. 20 to 70 years old

2. Minimum of 20 teeth, including two of the following experimental teeth in each quadrant:
   - First premolar
   - Second premolar
   - First molar
   - Second molar

3. 4 mm to 7 mm pocket depths on all experimental teeth

4. Absent from periodontal surgery post January 1, 1984

5. Patients in medical-risk American Society of Anesthesia (A.S.A.) categories I and II to be considered only.

   Those patients possessing any of the following will be eliminated from the study:

   a. Uncontrolled hypertensive disorders
   b. Uncontrolled diabetes
   c. Seizure disorders controlled with dilantin, phenobarbital, or their derivatives
   d. Requirement of prophylactic antibiotic coverage (i.e., valvular prosthesis, hip prosthesis)
   e. Sodium-restricted diets
   f. Hepatitis
   g. Venereal disease
   h. Tuberculosis
   i. A.I.D.S.
   j. Active Gastrointestinal disorders
   k. History of radiation to the head and neck region
   l. History of steroid therapy post January 1, 1984 for a minimum time period of two weeks
   m. Compromised immune system
   n. Hodgkin's Disease
   o. Chemotherapy
   p. Immunosuppressive agents

6. Absent from antibiotic coverage post November 1, 1984

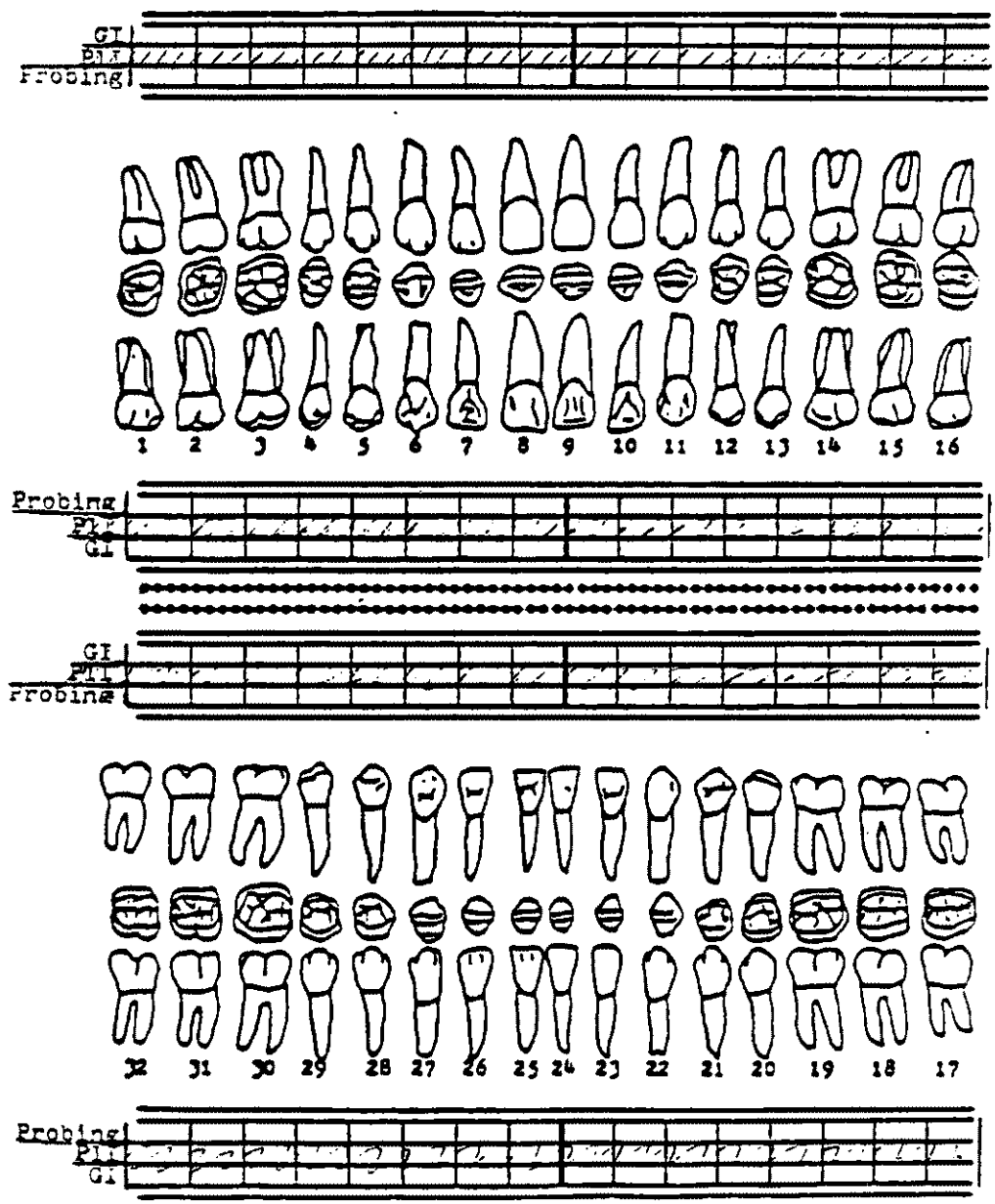
7. Free of mental or physical impairments that interfere with the performance of proper oral hygiene

APPENDIX M

INTRARATER RELIABILITY STANDARDIZATION CHART FOR PROBING AND GINGIVAL INDEX

INTRARATER RELIABILITY STANDARDIZATION CHART FOR PROBING AND GINGIVAL INDEX

PATIENT'S NAME _____ DATE _____



APPENDIX N

INTRARATER RELIABILITY STANDARDIZATION

MICROBIAL EVALUATION

INTRARATER RELIABILITY STANDARDIZATION
MICROBIAL EVALUATION *

Information:

Experimental Tooth Number:

3,4 (5 or 2, respectively, if either is absent)
13,14 (12 or 15,respectively,if either is absent)
19,20 (18 or 21,respectively,if either is absent)
29,30 (28 or 31,respectively,if either is absent)

Tooth surface:

DB = Distobuccal

B = Buccal

MB = Mesio-buccal

DL = Disto-lingual

L = Lingual

ML = Mesio-lingual

Tooth Utilized:

Deepest periodontal pocked initially detected among the experimentsl teeth in each quadrant.

Patient number _____

Patient Information	Bacteroides melaninogenicus	
Appointment Number	Tooth Number	
1		

*Designed Specifically for This Investigation

APPENDIX O

GENERAL STATISTICS FOR MICROBIAL DATA FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

General Statistics for Microbial Data
For the Four Treatment Methods
at Baseline

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	6.633	9.149	28.000
Control Dentifrice with Scaling and Root Planing	2.733	6.164	24.500
Experimental Dentifrice without Scaling and Root Planing	8.567	10.894	33.500
Control Dentifrice without Scaling and Root Planing	5.633	7.708	25.000

For each Treatment Method n=15

General Statistics for Microbial Data
for the Four Treatment Methods
at Week Four

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	0.800	1.486	4.500
Control Dentifrice with Scaling and Root Planing	0.900	1.442	5.000
Experimental Dentifrice without Scaling and Root Planing	2.333	4.078	14.500
Control Dentifrice without Scaling and Root Planing	1.267	2.329	7.000

For each Treatment Method n=15

General Statistics For Microbial Data
For The Four Treatment Methods
At Week Eight

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.467	3.782	13.500
Control Dentifrice with Scaling and Root Planing	0.633	1.674	6.000
Experimental Dentifrice without Scaling and Root Planing	0.800	1.667	5.500
Control Dentifrice without Scaling and Root Planing	0.433	1.294	5.000

For each Treatment Method n=15

APPENDIX P

GENERAL STATISTICS FOR GINGIVAL INDEX DATA FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

General Statistics For Gingival Index Data
For The Four Treatment Methods
At Baseline

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.300	0.389	2.500
Control Dentifrice with Scaling and Root Planing	1.342	0.319	2.125
Experimental Dentifrice without Scaling and Root Planing	1.492	0.449	2.375
Control Dentifrice without Scaling and Root Planing	1.450	0.448	2.750

For each Treatment Method n=15

General Statistic For Gingival Index Data
For The Four Treatment Methods
At Week Four

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.058	0.133	1.500
Control Dentifrice with Scaling and Root Planing	1.058	0.093	1.250
Experimental Dentifrice without Scaling and Root Planing	1.075	0.132	1.500
Control Dentifrice without Scaling and Root Planing	1.075	0.104	1.250

For each Treatment Method n=15

General Statistics For Gingival Index Data
For The Four Treatment Methods
At Week Eight

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.017	0.044	1.125
Control Dentifrice with Scaling and Root Planing	1.000	0.000	1.000
Experimental Dentifrice without Scaling and Root Planing	1.025	0.052	1.125
Control Dentifrice without Scaling and Root Planing	1.025	0.070	1.250

For each Treatment Method n=15

APPENDIX Q

GENERAL STATISTICS FOR PLAQUE INDEX DATA FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

General Statistics for Plaque Index Data
For The Four Treatment Methods
At Baseline

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.167	0.349	2.000
Control Dentifrice with Scaling and Root Planing	1.233	0.334	2.000
Experimental Dentifrice without Scaling and Root Planing	1.400	0.498	2.500
Control Dentifrice without Scaling and Root Planing	1.317	0.372	2.000

For each Treatment Method n=15

General Statistics For Plaque Index Data
For The Four Treatment Groups
At Week Four

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.000	0.000	1.000
Control Dentifrice with Scaling and Root Planing	1.017	0.065	1.250
Experimental Dentifrice without Scaling and Root Planing	1.033	0.088	1.250
Control Dentifrice without Scaling and Root Planing	1.000	0.000	1.000

For each Treatment Method n=15

General Statistics For Plaque Index Data
For The Four Treatment Groups
At Week Eight

Variable	Mean	Standard Deviation	Maximum Value
Experimental Dentifrice with Scaling and Root Planing	1.000	0.000	1.000
Control Dentifrice with Scaling and Root Planing	1.000	0.000	1.000
Experimental Dentifrice without Scaling and Root Planing	1.000	0.000	1.000
Control Dentifrice without Scaling and Root Planing	1.000	0.000	1.000

For each Treatment Method n=15

APPENDIX R

RAW DATA OF QUANTITATIVE MICROBIAL COLONIES FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

Raw Data of Quantitative Microbial Colonies For The Four Treatment Methods At Baseline, Week Four, And Week Eight

T1 = Treatment 1: Experimental Dentifrice with Scaling and Root Planing

T2 = Treatment 2: Control Dentifrice with Scaling and Root Planing

T3 = Treatment 3: Experimental Dentifrice without Scaling and Root Planing

T4 = Treatment 4: Control Dentifrice without Scaling and Root Planing

Subject	Treatment	Baseline	Week 4	Week 8
1	T1	0.0	0.0	0.0
	T2	1.0	2.5	0.0
	T3	0.0	3.5	0.0
	T4	2.0	1.5	0.0
2	T1	1.0	0.0	0.0
	T2	0.0	0.0	0.5
	T3	0.0	1.0	0.0
	T4	0.0	2.0	0.0
3	T1	4.0	1.0	13.5
	T2	4.0	1.5	6.0
	T3	6.0	0.0	0.0
	T4	25.0	1.5	0.0
4	T1	0.0	1.5	1.0
	T2	1.0	0.5	3.0
	T3	31.0	14.5	5.5
	T4	3.5	6.5	5.0

Subject	Treatment	Baseline	Week 4	Week 8
5	T1	2.0	0.0	0.0
	T2	3.5	5.0	0.0
	T3	1.0	0.0	0.0
	T4	17.5	0.5	0.0
6	T1	0.5	1.0	0.0
	T2	0.0	1.0	0.0
	T3	3.5	2.5	0.0
	T4	0.0	0.0	0.5
7	T1	9.5	0.0	0.5
	T2	2.0	0.5	0.0
	T3	17.5	4.5	3.0
	T4	8.5	0.0	1.0
8	T1	0.0	0.0	0.0
	T2	1.0	0.0	0.0
	T3	9.5	0.5	0.0
	T4	15.5	0.0	0.0
9	T1	22.0	4.5	7.0
	T2	2.5	0.0	0.0
	T3	33.5	0.0	0.5
	T4	5.5	7.0	0.0
10	T1	2.5	0.0	0.0
	T2	0.0	0.0	0.0
	T3	1.5	0.0	0.0
	T4	1.0	0.0	0.0

Subject	Treatment	Baseline	Week 4	Week 8
11	T1	0.0	0.0	0.0
	T2	1.5	2.5	0.0
	T3	13.5	0.0	3.0
	T4	0.0	0.0	0.0
12	T1	28.0	4.0	0.0
	T2	0.0	0.0	0.0
	T3	4.0	0.0	0.0
	T4	0.5	0.0	0.0
13	T1	18.0	0.0	0.0
	T2	24.5	0.0	0.0
	T3	1.5	8.0	0.0
	T4	0.0	0.0	0.0
14	T1	11.0	0.0	0.0
	T2	0.0	0.0	0.0
	T3	5.5	0.5	0.0
	T4	3.5	0.0	0.0
15	T1	1.0	0.0	0.0
	T2	0.0	0.0	0.0
	T3	0.5	0.0	0.0
	T4	2.0	0.0	0.0

APPENDIX S

RAW DATA OF GINGIVAL INDEX VALUES FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

Raw Data Of Gingival Index Values For The Four Treatment Methods At Baseline, Week Four, And Week Eight

T1 = Treatment 1: Experimental Dentifrice with Scaling and Root Planing

T2 = Treatment 2: Control Dentifrice with Scaling and Root Planing

T3 = Treatment 3: Experimental Dentifrice without Scaling and Root Planing

T4 = Treatment 4: Control Dentifrice without Scaling and Root Planing

Subject	Treatment	Baseline	Week 4	Week 8
1	T1	1.000	1.000	1.125
	T2	1.375	1.125	1.000
	T3	1.000	1.125	1.000
	T4	1.125	1.125	1.125
2	T1	1.125	1.125	1.000
	T2	1.625	1.250	1.000
	T3	1.625	1.125	1.125
	T4	1.625	1.000	1.250
3	T1	1.000	1.000	1.000
	T2	1.375	1.000	1.000
	T3	1.000	1.000	1.000
	T4	1.000	1.000	1.000
4	T1	2.500	1.500	1.125
	T2	2.125	1.250	1.000
	T3	2.250	1.500	1.125
	T4	2.750	1.250	1.000

Subject	Treatment	Baseline	Week 4	Week 8
5	T1	1.125	1.000	1.000
	T2	1.375	1.000	1.000
	T3	1.250	1.000	1.000
	T4	1.125	1.000	1.000
6	T1	1.125	1.000	1.000
	T2	1.000	1.000	1.000
	T3	1.375	1.125	1.125
	T4	1.875	1.250	1.000
7	T1	1.250	1.000	1.000
	T2	1.375	1.000	1.000
	T3	1.500	1.000	1.000
	T4	1.625	1.250	1.000
8	T1	1.250	1.000	1.000
	T2	1.250	1.000	1.000
	T3	1.500	1.000	1.000
	T4	1.625	1.125	1.000
9	T1	1.125	1.000	1.000
	T2	1.250	1.000	1.000
	T3	1.500	1.125	1.000
	T4	1.125	1.000	1.000
10	T1	1.125	1.000	1.000
	T2	1.000	1.000	1.000
	T3	1.375	1.000	1.000
	T4	1.125	1.000	1.000

Subject	Treatment	Baseline	Week 4	Week 8
11	T1	1.500	1.125	1.000
	T2	1.750	1.125	1.000
	T3	1.500	1.125	1.000
	T4	1.375	1.000	1.000
12	T1	1.750	1.125	1.000
	T2	1.000	1.000	1.000
	T3	2.125	1.000	1.000
	T4	1.375	1.000	1.000
13	T1	1.375	1.000	1.000
	T2	1.125	1.000	1.000
	T3	1.000	1.000	1.000
	T4	1.500	1.000	1.000
14	T1	1.000	1.000	1.000
	T2	1.500	1.125	1.000
	T3	2.375	1.000	1.000
	T4	1.500	1.125	1.000
15	T1	1.250	1.000	1.000
	T2	1.000	1.000	1.000
	T3	1.000	1.000	1.000
	T4	1.000	1.000	1.000

APPENDIX T

RAW DATA OF PLAQUE INDEX VALUES FOR THE FOUR TREATMENT METHODS AT BASELINE, WEEK FOUR, AND WEEK EIGHT

Raw Data Of Plaque Index Values For The Four Treatment Methods At Baseline, Week Four, And Week Eight

T1 = Treatment 1: Experimental Dentifrice with Scaling and Root Planing

T2 = Treatment 2: Control Dentifrice with Scaling and Root Planing

T3 = Treatment 3: Experimental Dentifrice without Scaling and Root Planing

T4 = Treatment 4: Control Dentifrice without Scaling and Root Planing

Subject	Treatment	Baseline	Week 4	Week 8
1	T1	1.00	1.00	1.00
	T2	1.75	1.00	1.00
	T3	1.00	1.00	1.00
	T4	1.75	1.00	1.00
2	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.25	1.00
	T4	1.00	1.00	1.00
3	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.00	1.00
	T4	1.00	1.00	1.00
4	T1	2.00	1.00	1.00
	T2	2.00	1.00	1.00
	T3	2.00	1.00	1.00
	T4	2.00	1.00	1.00

Subject	Treatment	Baseline	Week 4	Week 8
5	T1	1.25	1.00	1.00
	T2	1.25	1.25	1.00
	T3	2.00	1.00	1.00
	T4	1.50	1.00	1.00
6	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.00	1.00
	T4	1.00	1.00	1.00
7	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.25	1.00
	T4	1.00	1.00	1.00
8	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.25	1.00	1.00
	T4	1.25	1.00	1.00
9	T1	1.25	1.00	1.00
	T2	1.50	1.00	1.00
	T3	2.00	1.00	1.00
	T4	1.50	1.00	1.00
10	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.25	1.00	1.00
	T4	1.00	1.00	1.00

Subject	Treatment	Baseline	Week 4	Week 8
11	T1	1.00	1.00	1.00
	T2	1.50	1.00	1.00
	T3	1.50	1.00	1.00
	T4	1.50	1.00	1.00
12	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.50	1.00	1.00
	T4	1.25	1.00	1.00
13	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.00	1.00
	T4	1.00	1.00	1.00
14	T1	2.00	1.00	1.00
	T2	1.50	1.00	1.00
	T3	2.50	1.00	1.00
	T4	2.00	1.00	1.00
15	T1	1.00	1.00	1.00
	T2	1.00	1.00	1.00
	T3	1.00	1.00	1.00
	T4	1.00	1.00	1.00