The Anti-Inflammatory Effect of Colgate Total® Toothpaste on Microbial Pathogens during an Experimental Gingivitis Model

by

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Dedication

To my mentor Janet Kinney, whose enthusiasm for clinical research inspired me to pursue this experimental gingivitis study as my thesis project.

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CHAPTER I

INTRODUCTION

1.1 Problem Statement

Oral inflammatory disease in the United States is widespread, with the vast majority of adults exhibiting clinical manifestations of gingivitis or periodontitis.[1] It is well known that microbial pathogens are an integral component of the pathogenesis of periodontal diseases.[2] Approximately forty years ago, the concept that bacteria functions as a biofilm community began to emerge.[1] Further evidence has revealed the complex organization of biofilm and the corresponding relationship between the colonies.[1] Oral plaque biofilm balance is attributed to the transition from oral health to disease, and the pathogenicity of biofilm is inherently reduced through effective oral hygiene.[3]

Gingivitis is typically diagnosed through the observance of inflammation in the gingival tissue with the absence of clinical attachment loss.[4] Inflammation is the innate reaction of the human body to cellular injury, and oral inflammation is characterized by changes in gingival color, contour, and consistency.[5] If gingival inflammation is not reversed through improved oral hygiene or professional prophylaxis, coronal alveolar bone resorption and damage to the junctional epithelium resulting from the apical shift and breakdown of collagen fibers develops, and the subsequent development of periodontal disease occurs.[4]

Similar to natural gingivitis, experimental gingivitis is the result of microbial plaque formation.[6,7] In clinical research, the experimental gingivitis model is used to evaluate the resultant inflammatory response to the oral microflora as the process of moving from gingival health to gingivitis occurs in a controlled environment.[7]

Emergent evidence of a relationship between biofilm, inflammation, oral health, and systemic diseases such as cardiovascular disease, diabetes, and chronic respiratory disease affirms the importance of maintaining proper oral health.[5] Colgate Total® toothpaste contains triclosan, which is a broad spectrum antimicrobial, and a polyvinylmethyl ether maleic acid copolymer (PVM / MA) binder to increase the uptake and retention of triclosan to the enamel and buccal epithelium.[8] This unique formulation has been shown to provide up to 12 hours of antimicrobial protection, invariably inhibiting biofilm growth.[5,8] Furthermore, it has also been shown to be effective at reducing plaque accumulation and subsequent gingival inflammation, potentially restricting bacterial infection and the progression of periodontal disease.[5,8] At the present time, Colgate Total® is the only dentifrice formulated with triclosan that is available for use in the United States.[8]

A comprehensive review of the literature revealed several previous clinical studies that have focused on the effects of triclosan containing dentifrices on the oral microflora.[9–12] A meta-analysis of 16 studies comparing Colgate Total® to a fluoride toothpaste determined that Colgate Total® did significantly reduce gingival bleeding and improve gingival health.[10] However, there is a lack of evidence in the literature to support the effects of Colgate Total® toothpaste on microbial pathogens in the total absence of mechanical interruption of plaque biofilm. The results of this research are

important because they provide evidence of the reduction of pathogenic microbes in the oral cavity in the absence of mechanical plaque removal. The results of this study may improve the oral health of those who are incompliant with oral hygiene recommendations, and those with compromised manual dexterity such as the elderly, arthritic, or the developmentally disabled.

1.2 Goal Statement

The goal of this proposed study was to conduct a pilot, randomized, controlled, clinical trial to determine the effect of Colgate Total® toothpaste on microbial pathogens in plaque biofilm samples during an experimental gingivitis model.

1.3 Specific Aims

The purpose of this clinical research study was to:

1) Determine the effect of Colgate Total® toothpaste on microbial pathogens in plaque biofilm samples during an experimental gingivitis model in the absence of mechanical interruption of plaque biofilm.

With respect to the study objective, the following alternative hypothesis was constructed:

1a) Colgate Total® toothpaste, as compared to a standard of care toothpaste, will be more effective in reducing microbial pathogens in plaque biofilm samples during the experimental gingivitis model.

1.4 Significance

Although evidence exists to support the clinical efficacy of Colgate Total® toothpaste, little is known of the effect of this dentifrice on microbial pathogens. The results of this study provide evidence of the true effect of Colgate Total® toothpaste on gingival health and plaque biofilm samples in the absence of mechanical plaque biofilm removal during an experimental gingivitis model, and substantiate the need for further research on this topic.

1.5 Thesis Overview

A detailed overview is presented to facilitate insight of this thesis. A comprehensive Review of the Literature is depicted in Chapter II, and is divided into twelve primary subsections, including the history of microbial pathogens, the concept of bacteria as biofilm, biofilm development in the oral cavity, oral diseases associated with biofilm, natural vs. experimental gingivitis, indices for assessing plaque and gingival inflammation, microbial analysis, Colgate Total® toothpaste, triclosan, polyvinylmethyl ether maleic acid copolymer, clinical efficacy of Colgate Total® toothpaste, and triclosan dentifrice / mouthrinse formulations available in other countries. Chapter III outlines the Materials and Methods utilized during the study, and the subsequent Results of the statistical analysis are presented in Chapter IV. This section is followed by Chapter V, the Discussion and interpretation of the study results, and the thesis Conclusion is portrayed in Chapter VI.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 History of Microbial Pathogens

Microbial pathogens are a fundamental component of the pathogenesis of periodontal disease.[2] The initial concept of a relationship between microbial pathogens and infectious disease occurred more than 480 years ago through the work of the Italian physician Girolamo Fracastoro.[13] His theories involving the source of syphilis infections set the precedent for one of the critical junctures in the history of microbes, the germ theory of disease.[13]

The distinctive and pervasive nature of microbes was affirmed through the invention and advancement of microscopy.[14] Plaque microorganisms were first observed microscopically in the late 17th century by the Dutch botanist Anton van Leeuwenhoek, initiating the concept of a relationship between microbes and infectious disease.[1,2,14] His primitive microscope was similar to the performance of present day light microscopy, and provided unique visualization which sustained the first evidence of the etiologic determinants of infectious disease.[14] Furthermore, this advance in technology provided a rationale for scientists to establish the etiology of several systemic diseases.[15]

The next century yielded the development of the vaccination process by William Jenner, when he discovered that milkmaids in contact with cowpox were immune to smallpox infection.[13] Although he lacked knowledge of the biological means of withstanding disease, or the host's immune response to another disease agent, what began as an experiment emerged as an enduring method of preventing disease transmission.[2] In the 1970's, the last cases of smallpox were reported, and this disease became the first infection to be completely eliminated from humanity.[13]

In the late 19th century, the germ theory of disease was developed through the work of Louis Pasteur, a chemist, and Robert Koch, a German professor of public health, providing a novel approach for observing and combatting disease, and yielding confirmation that bacteria indeed induces disease.[13] Through his scientific work, Pasteur unveiled the broad existence of microbiology, and invented the process of pasteurization, which destroyed microbes in milk, and prevented the spread of infectious disease.[13] His determination that bacteria is the etiology of disease led to the breakthrough of surgeon Joseph Lister's use of carbolic acid as an antiseptic during procedures.[13,15] In addition, he is credited with the development of new vaccines, including those to treat rabies and anthrax.[13] Koch's primary contribution was through a process he developed known as "Koch's Postulates," which were composed of four developmental and analytical measures to determine if a microbe was related to a specific disease.[13,15] His assumption was that through eliminating or decreasing pathogens, the disease progression may cease or at a minimum be reduced.[15] He also formulated the development of solid nutrient media, which unveiled the use of pure culture to isolate organisms.[16] His work led to the identification of the bacteria known to

cause tuberculosis, anthrax, wound infections, and cholera, which increased awareness among his students and competitors in the quest for relating microbes to their resultant disease.[13,14]

The turn of the century marked the continued emergence of the existence of microbiology.[13] In the late 1890's, Dmitri Ivanowski, a Russian microbiologist, and Martinus Beijerinck, a Dutch botanist, detected unknown microbes uninhibited by filters used to block the passage of bacteria, which were named "filtrable viruses".[13] The study of microbiology then embarked on a new path, with many researchers using scientific theory to determine the pathogenesis of disease and modes of prevention.[13] However, many biologist were skeptical due to the tiny size of the microbes.[13] In the 1930's, advancements in microscopy revealed the complex structure of bacterial cells, and biologists began to show interest in microbiology; consequently, these separate entities began to unite.[13]

In 1929, the discovery of penicillin in mold by Alexander Fleming provided an innovative approach to combat bacterial disease.[13] During the 1960's, advances in medicine due to new antibiotics and vaccines increased the presumption of infectious disease control and treatment through the subjugation of microbial pathogens.[13] Expanded knowledge of bacteria and viruses led to the determination of pathogenic versus probiotic microbes, and the notable increase in life expectancy was largely attributed to these microbiological advances and the development of antibiotics.[13] However, the discovery new infectious diseases such as the Acquired Immune Deficiency Virus, Human Immunodeficiency Virus, and Hepatitis Virus revealed this was not the case.[13] These viruses expanded further the field of biomedical and

microbiological research, and have led to a close and continual evaluation of our existence with microbes.[13]

2.2 Concept of Bacteria as Biofilm

In the 1970's, the concept of bacteria functioning as biofilm began to emerge.[1] The next two decades conveyed evidence of the complex organization of attached bacteria, and how the combination of different microbes constitutes bacterial plaque.[1] Approximately ninety-five percent of all bacteria in nature constitutes as biofilm.[17] A biofilm is described as an aggregation of microbiological cells encompassed in a sessile matrix community, that thrives on neighboring accessible nutrients for survival and growth.[5,6] Different microbes are known to frequently combine with one another, and then further combine with additional microbes to generate a substantial bacterial mass.[1] Microscopic evidence has revealed the intricate organization of biofilms, with small surface colonies proliferating into structured, larger colonies that collaborate with one another.[1]

Numerous chronic bacterial infections are composed of biofilms, and are often not readily treated through conventional antibiotic therapy. [1] In addition, approximately half of all infectious diseases that are found in slightly compromised hosts can be related to organisms that are located in the environment or the human body.[1] Different biofilms share common clinical characteristics, including a preference for inert surface or dead tissue, but can also form on living tissue.[1] Furthermore, biofilms tend to grow at a slow rate in multiple locations, and can be slow to produce symptoms; as a result, biofilms are uncommonly resolved through the host defense mechanisms, and this is true even

for individuals with healthy immune systems.[1] Although antibiotics have been shown to decrease the planktonic cells released from the biofilm, they fail to completely destroy the bacteria by failing to completely penetrate the biofilm, resulting in recurring symptoms until the biofilm is detached from the body.[1]

2.3 Biofilm Development in the Oral Cavity

Once supragingival plaque accumulates, a unique biofilm consisting of motile organisms and gram-negative, anaerobic microbes, manifests subgingivally.[18] The biofilm is by definition attached to a surface, whether it be a tooth, host-mediated material adjacent to the tooth, the epithelium, or other microbes that have adhered to these areas.[18] The interactions between these organisms have a vital function to their survival and growth.[18] Emergent evidence has shown through recent microbial ecosystem diversity analysis, a new perspective of the transition from health to disease is related to a change in the overall balance of the oral microflora, instead of the presence of specific individual periodontal pathogens.[19] Increased understanding of the oral microbiome is important to dentistry because through improved diagnosis, clinicians may be able to determine if periodontal disease is present before clinical signs are manifested.[19] Although it is undesirable and impossible to completely eliminate dental biofilm, the pathogenicity can be diminished through bioburden reduction and proper oral hygiene.[17] Current research is focusing on the molecular and genetic structure of biofilm formation, and comprehensive systems-level analysis of the relationship between the host and the microbiome.[19] Classification of the putative pathogens associated with oral biofilm has been arduous, despite thorough analysis of the microbes affiliated with oral disease.[20] Oral biofilms are distinguished, readily

attainable, and contain a paramount affiliation with disease and health in humans.[20] Although approximately half of the oral microbial flora can be cultured through standard means, there are many organisms that are impossible to culture, which inhibits comprehensive discernment of oral biofilm compostition.[20] Once the oral biofilm attaches to a surface, the flora endures in a dynamic, "microbial homeostasis."[20] The biofilm customarily exists together with its host, encouraging the host defense against invading pathogenic microbes, and creating the subsequent physiologic host response.[20] Periodically, there may be a disruption in the biofilm-host association due to environmental conditions including poor oral hygiene, dietary intake, salivary flow rate, and the host defense mechanisms, resulting in oral disease.[20,21]

2.4 Oral Diseases Associated with Biofilm

The most prolific oral diseases, including enamel caries, periodontitis, and gingivitis, are associated in part with biofilm formation.[16] The progression of oral disease development is episodic, and is detrimental to the social, economic, and physical well-being of those afflicted with these diseases. [22] Oral disease is widespread, with socioeconomic status inherently related to oral disease risk, and those living in poverty disproportionately affected.[22,23]

Globally, the World Health Organization estimates that dental caries effects between sixty and ninety percent of all school aged children, and the vast majority of adults.[23] Furthermore, severe forms of periodontal disease affect fifteen to twenty percent of adults between the ages of 35 and 44, and approximately thirty percent of individuals between the ages of 65 and 74 are completely edentulous.[23]

In the United States, almost all adults demonstrate clinical symptoms of gingivitis or periodontitis, with severe forms of periodontitis affecting about fourteen percent of those between the ages of 45 to 54, and increasing to twenty-three percent for those between the ages of 65 and 74.[22] Although decreasing, the number of edentulous U.S. adults is approximately thirty percent.[22] Dental caries is the most common disease in American children, and is five times more common than asthma.[22]

Maintaining oral health is crucial to overall health and well-being.[22,23] Dental caries, periodontal disease, and gingivitis are preventable through patient education, proper oral hygiene, a healthy diet of fruits and vegetables, decreasing sugar consumption, fluoride exposure, and avoidance of alcohol and tobacco use.[22,23] In addition, the use of a dentifrice with anticaries and antigingivits properties such as Colgate Total® may prove beneficial for oral disease prevention and overall health promotion.

a. Dental Caries

Dental caries is the most common childhood disease, with more than seventy percent of children in the United States diagnosed with caries by the age of seventeen.[24] Caries development is multifactorial, and results from acidic products of bacterial fermentation of carbohydrates, leading to the breakdown of enamel and dentin.[25] This breakdown occurs through a combination of established oral bacterial biofilm on a tooth surface, and the oral environment balance.[25] The oral environment is dependent on several aspects of the oral cavity, including the individuals dietary intake (particularly sugar), amount of fluoride in plaque and enamel, buffer capacity, flow rate and viscosity of saliva, and the quantity and types of microbes present.[25] Once the pH

reaches a critical level, demineralization of the tooth structure begins, and can invariably progress through enamel, the dentin, and reach the pulp.[25] If left untreated, the infection can subsequently spread to the oral tissues and alveolar bone.[25]

Microbes Affiliated with Dental Caries

The most prominent pathogenic microbes associated with dental caries are *Streptococcus mutans* and *Lactobacillus*.[25] *S. mutans* is theorized to be the primary etiologic microbe associated with dental caries, with *Lactobacillus* more prominent in secondary development, including dentinal caries.[25] Both of these microorganisms thrive in acidic environments, and have the capability to quickly metabolize sugars to acid.[25] As the caries proliferate into dentin, the microbial composition becomes more complex, and includes species from *Bifidobacterium, Parvimonas, Rothia, Actinomyces, Eubacterium,* and *Lactobacillus*.[25]

16S rRNA molecular studies of advanced caries revealed a preponderance of *S. mutans* and *Lactobacillus* organisms, with the presence of *Selenomonas, Fusobacterium, Psuedoramibacter, Prevotella, Dialister,* and *Bifidobacterium* also noted.[25] Caries progression is dependent on the amount and frequency of carbohydrate exposure, in particular dietary glucose and sucrose, and the tooth's vulnerability to caries development, such as the occlusal surface of molars.[25] Furthermore, the amount of *Lactobacillus* organisms found in the oral flora is relative to carbohydrate exposure.[25] Current research has also focused on detecting possible genetic links relative to caries development.[25]

b. Periodontal Disease

Periodontal disease is a common oral infection characterized by clinical attachment loss, alveolar bone breakdown, and chronic inflammation associated with an increased systemic inflammatory response.[26] Gram-negative, anaerobic oral bacterial biofilms are often found in individuals with periodontal disease.[1] Over the past three to four decades, extensive research has provided evidence that the etiology of periodontal disease is predominantly related to plaque pathogens.[18,27] In addition to these pathogens, the observable traits and the rate of disease progression are dependent on both genetic, lifestyle, and acquired components, which are important determinants of developing plaque induced periodontal disease infection.[4]

Classifications of Periodontal Disease

There are seven classifications of periodontal disease, including: 1) gingivitis, 2) chronic periodontitis, 3) aggressive periodontitis, 4) periodontitis as a manifestation of systemic disease, 5) necrotizing periodontal disease, 6) abscesses of the periodontium, and 7) periodontitis associated with endodontic lesions.[4] Each of these classifications has the potential to progress, albeit acutely or chronically, and be unresponsive to active treatment.[4] Because the mode of progression from gingivitis to periodontitis has not been substantiated, the exact mechanism of initial periodontal disease induction is unknown.[28] However, environmental and lifestyle factors such as smoking, uncontrolled diabetes, and lack of patient compliance have been shown to contribute to disease recurrence.[29] Furthermore, it has been established that symptoms of gingivitis

can manifest in individuals previously diagnosed with periodontal disease that currently appear to be stable.[4]

Non-Specific vs. Specific Plaque Hypothesis

There are two theories for determining the etiology of periodontal disease progression, the non-specific and specific plaque hypothesis.[15] Initially, the non-specific plaque hypothesis was widely accepted.[18] The essence of this theory is that all microorganisms are inherently associated with periodontal disease, oral diseases are the result of plaque mass accumulation, and inflammation is prevented and eliminated through the complete mechanical removal of plaque bacteria.[30] In addition, this theory disregards any possibility that the diverse structure of bacterial colonies is relative to pathogenicity, and fails to substantiate why some areas of gingival inflammation fail to progress into periodontitis.[30] This hypothesis fell out of favor as evidence emerged of the qualitative differences between oral plaque during periodontal health and disease.[18]

The specific plaque hypothesis is based on the concept that certain plaque pathogens are the etiologic agents of periodontal disease, with the individual inflammatory and systemic immune response as the basis for cellular difficulty.[15] As the specific plaque hypothesis became apparent, emergent measures for determining periodontal pathogens were incorporated, and include the host immune response, virulence, association, elimination of pathogens, risk assessment, and animal studies.[18]

Once it was determined that the oral microbial community primarily consisted of gram-positive microorganisms, and then shift to primarily gram-negative composition as the plaque accumulates and inflammatory processes proliferate, Socransky and

associates developed the concept of five “complexes” for the subgingival microflora, based on associations of specific groups of bacteria with each other and with disease status.[15] This approach ascertained that the microbiological environment is generated by the individual microbes, and that the relationship between the microbes determines the extent of disease.[15]

Host Immune Response

The host immune response is beneficial due to the production of antibodies aimed at the species, which function distinctly with the putative pathogen.[18] In addition, the ability of the pathogen to initiate disease may also be useful in determining pathogenicity.[18] The response of the human immune system is generally classified as either innate or adaptive (acquired) immunity.[31] Both of these immune responses, combined with physiological and anatomical barriers (such as salivary lysozymes and intact skin), protect the human host from invading pathogens.[31]

The innate immune system begins the inflammatory response shortly after the invading pathogen attacks the host.[31] The innate response utilizes a minor amount of invariant receptors to locate infectious pathogens, and offsets this by focusing on large numbers of pathogens with common microbial elements.[31] In addition to the pivotal role it plays in the host defense mechanism to invading infectious pathogens, innate immunity has a decisive role in the regulation of the inflammatory response due to human disease, and enhances the defense provided by the physiological and anatomical barriers.[31]

The adaptive immune response is generated after the initial innate immune response to the invading pathogen.[31] Antigen exposure forms antibodies and the subsequent adaptive response, with T- and B- lymphocytes functioning as the primary defense mechanisms.[31] A large variety of randomly chosen receptors allow the adaptive immune response to identify numerous antigens, which differs from the small amount of receptors used in the innate response.[31] Although innate immunity occurs in all multicellular species, adaptive immunity is formed only in complex vertebrates and jawed fish.[31] The host's defense to the invading pathogen is accomplished through collaboration of both the innate and adaptive immune systems.[31]

Microbes Affiliated with Periodontal Disease

The microbes affiliated with periodontal disease include *A. actinomycetemcomitans, B. forsythus, P. micros, P. gingivalis, S. intermedius, C. rectus, P. intermedia, E. nodatum,* and *Treponema sp*.[2,18] These organisms are prevalent in both gingivitis and periodontitis, but are more prolific in subjects with advanced periodontitis.[32] Ramseier and colleagues conducted a clinical study to determine if putative host- and microbially derived biomarkers are capable of identifying the presence of periodontal disease using plaque biofilm and whole saliva.[33] Ninety-nine human subjects were equally recruited into a healthy/gingivitis group or a periodontitis group and divided into four subgroups prior to data analysis.[33] Subgingival plaque biofilm species were analyzed including *A. actinomycetemcomitans, C. rectus, F. nucleatum, P. intermedia, P. gingivalis, T. forsythia, E. corrodens, and T. denticola.*[33] When comparing the healthy/gingivitis group to the periodontal pathogen group, biomarker data derived from the plaque pathogen samples revealed that *T. denticola, P. gingivalis, T. forsythia, P. intermedia,* and *C.*

rectus demonstrated significant differences ($p < 0.001$), whereas *F. nucleatum and E. corrodens* did not.[33] The ability of these pathogens to identify periodontal disease was greater when paired with the salivary biomarkers, which included MMP-8, MMP-9, Calprotectin, IL-Iβ, ICTP, IL-6, and TNF-α, among others.[33] In addition to the pathogenic microbes in the oral flora, the individual systemic immune response, smoking status, and systemic disease are vital components in the development and progression of gingivitis and periodontitis.[34]

There is limited knowledge to date of microbial succession in supra and sub gingival plaque in both healthy and diseased subjects, and the identification of specific time periods of bacterial pathogen colonization and growth would aid clinicians in the prevention and management of periodontal disease.[35] A recent study by Teles and colleagues evaluated the early ecological succession of bacterial organisms throughout seven days of abstinence from oral hygiene after professional removal of sub and supragingival plaque from both healthy subjects and subjects with periodontal disease, to determine if the species return at similar rates in both groups.[35] Overall, the subgingival plaque samples demonstrated fewer significant differences in the proportions of the organisms, which suggests that the subgingival ecosystem may take more time to redevelop, which may be due in part to the bacterial cells that were not removed during professional cleaning.[35] Significant changes in subgingival biofilm development were noted at later time points in the periodontal patients when compared to the healthy patients, but by day seven, more significant changes were observed in the periodontitis group.[35] Although the biofilm mass returned within days following the

cleaning, the climax community customary of the supra and subgingival tooth surfaces was not fully reestablished over the course of this study.[35]

The Relationship between Periodontal Disease and Systemic Disease

The detrimental effect of periodontal disease in the oral cavity is well documented. The prevalence of periodontal disease is widespread, with approximately 80 percent of people in the United States exhibiting some form of periodontal disease.[24] Emergent evidence of a relationship between oral health and systemic diseases such as cardiovascular disease, diabetes mellitus, and chronic respiratory disease affirms the importance of maintaining proper oral health.[3,5,36,] Currently, over 27 million adults in the United States are diagnosed with cardiovascular disease, 26 million are diagnosed with diabetes mellitus, and 15 million are diagnosed with chronic obstructive pulmonary disease.[37–39] Inflammatory mediators including interlukin-1 (IL-1), IL-6, and C-reactive protein have a suspected role in generating systemic inflammation.[3] Researchers continue to explore the relationship between periodontal disease and systemic disease, and additional information may yield an explanation to substantiate the extent of the biological basis of this association.[5]

Cardiovascular Disease

Cardiovascular disease is defined as the accumulation of inflammatory plaques that can lead to thrombosis and future myocardial infarction.[5] There are several theories to explain the statistical association between cardiovascular disease and periodontal disease, and include risk factors common to both conditions (such as tobacco use), or a specific consequence related to periodontal disease.[40] In addition, evidence has

implicated inflammation, systemic infection, and autoimmunity induction as potential components of the pathophysiology relative to these two diseases.[40] Atherosclerosis is an inflammatory condition that results from plaque accumulation in the arteries from cardiovascular disease.[5] Initial atherosclerotic plaques are comprised of neutrophils, monocytes and lymphocytes.[5] Once monocytes become macrophages, inflammation is generated by inflammatory mediators, and the plaque enlarges, possibly causing thrombosis or myocardial infarction.[5] Analysis of atherosclerotic plaque lesions found in carotid arteries revealed that 40 percent demonstrated antigens consistent with periodontal pathogens including *P. gingivalis, P. intermedia*, and *T. forsythia*.[5] Furthermore, platelet aggregation can be induced by *P*. *gingivalis*, suggesting a possible mechanistic relationship between oral microbial pathogens and atherosclerosis.[5]

Joshipura et al conducted a cross-sectional evaluation of data taken from the prospectional Health Professional Follow-up Study (HPFS) investigation of 468 men to evaluate the relationship between periodontal disease, tooth loss, and specific biomarkers in blood.[26] The results revealed an association between periodontal disease and elevated serum levels of biomarkers of endothelial dysfunction and dyslipidemia including CRP, t-PA, and LDL-C, which are known to be risk factors for cardiovascular disease.[26] Men without periodontal disease had a 30 percent lower level of CRP, and the biomarkers t-PA, vWF, and LDL-C were 11 percent lower when compared to men with periodontal disease.[26] A meta-analysis of 29 observational studies found that subjects with periodontal disease had a 34 percent higher risk of developing cardiovascular disease than those without diagnosed periodontal disease.[40] This

analysis provides further evidence of a relationship between oral health and systemic disease.

Diabetes Mellitus

Diabetes mellitus is described as a group of systemic conditions defined by a heightened quantity of blood glucose, which are associated with periodontal disease.[5,41] The relationship between these conditions is two-fold, with periodontitis shown to exacerbate poor glycemic control in diabetics when compared to subjects without diabetes, and diabetes increasing the risk of periodontitis.[5,41] It is theorized that because of an increase in systemic inflammation, diabetics are prone to infection, and bactericidal cell activity is inhibited; therefore, the prevalence and extent of periodontal disease devastation in diabetic subjects is elevated.[5] In addition, the inflammatory reaction to periodontal pathogens in diabetics is thought to encourage the development of periodontal disease.[41]

There have been numerous studies that provide evidence of the oral-systemic link between diabetes and periodontitis. In a meta-analysis of 48 clinical studies by Taylor, 44 studies (seven prospective and 37 cross-sectional) revealed evidence of diabetes as a risk factor for periodontal disease.[42] Furthermore, an examination of the data from the National Health and Nutrition Examination Survey (NHANES) III by Tsai et al of 4,343 individuals found that those with poorly controlled diabetes mellitus had a significantly greater prevalence of severe periodontal disease than those without diabetes.[43] The results of these studies provide further evidence of the relationship between periodontal disease, inflammation, and systemic disease.

Chronic Respiratory Disease

In addition to cardiovascular disease and diabetes mellitus, systemic lung diseases such as chronic obstructive pulmonary disease (COPD) and nosocomial pneumonia infection have also been associated with periodontal disease.[5,36] COPD is widespread, ranking third on the list of death causes in the United States in 2011, and resulting in the loss of over 120,000 lives annually.[22,39] The resultant airflow obstruction is irrevocable, and leads to the disability of many individuals.[22]

Several recent studies have found a connection between oral health and chronic respiratory disease.[3,44] Scannapieco and Ho reviewed data from the NHANES III study and found a possible relationship between periodontal clinical attachment loss severity and individuals with COPD, with those having both periodontal disease and COPD exhibiting greater attachment loss when compared to those with periodontal disease without COPD.[45] In addition, the results appeared to reveal a decrease in lung function in those with COPD relative to the degree of clinical attachment loss.[45] A case-control study of 634 individuals conducted by Wang and colleagues determined a significant association between periodontal health and COPD.[3,44] An additional cross-sectional study of 392 subjects by Liu et al revealed an association between some indicators of periodontal health and COPD exacerbations.[3] Furthermore, a 25 year longitudinal of 1,118 men found that alveolar bone loss significantly elevated the risk of COPD, and the degree of severity of alveolar bone loss increased the risk of COPD throughout the follow-up period.[46] The results of these studies substantiate the need for further exploration of the relationship between periodontal disease as it compares to known causes of chronic respiratory disease such as smoking.[36]

c. Gingivitis

Gingivitis is the initial inflammatory response to microbial plaque accumulation, and is the only reversible classification of periodontal disease**.** [4] Gingivitis is widespread; it is estimated that more than fifty percent of all Americans exhibit clinical signs of gingivitis.[36] Plaque accumulation occurs within hours of the absence of oral hygiene, and the process of moving from gingival health to disease occurs within a matter of days.[36] Once adequate oral hygiene resumes, the clinical signs of gingivitis rapidly diminish.[47]

Gingivitis is diagnosed during a periodontal examination by the observance of inflammation in the gingival tissue with the absence of clinical attachment loss.[4] Inflammation is the innate reaction of the human body to cellular injury, and is marked by changes in color (redness), contour (swelling) and consistency, often exhibiting heat and pain.[5] Gingival inflammation can then progress, resulting in coronal alveolar bone resorption, which, combined with the breakdown and apical shift of collagen fibers from the junctional epithelium and cementum, leads to periodontal disease.[4]

2.5 Natural vs. Experimental Gingivitis

Both natural and experimental gingivitis develop due to an aggregation of microbial plaque.[6,7] The experimental gingivitis model differentiates from natural gingivitis in that it was established to evaluate both the initiation and resolving of gingival inflammation during a limited period of time, while being monitored by trained and calibrated examiners.[7] Subjects are chosen from a pool of volunteers who are free of periodontal and systemic disease, and meet the inclusion and exclusion criteria.[7] The treatment

area is covered with a fabricated stent similar to an occlusal guard, and the subjects refrain from brushing this area during the 21 day study period.[7] Clinical measurements including plaque and gingival crevicular fluid samples, gingival and plaque indices, and saliva samples are assessed at baseline, on specific days throughout the 21 day study period, and on the day 35 follow up visit.[7] After the 21 day test period is complete, the subject receives a thorough prophylaxis to remove the plaque and calculus accumulation.[7] Although the composition of microbes affiliated with natural gingivitis differ from experimental gingivitis (see sections 2.5 a. and 2.5 d.), the experimental gingivitis model is useful for studying inflammation as it reacts to the increase in microbial plaque accumulation, without causing permanent adverse effects to periodontium.[7]

a. Natural Gingivitis

As microbial plaque aggregates, colonization begins and the flora becomes more complex, and gingivitis is induced.[6] Clinical characteristics of gingivitis are intermittent, consisting of bouts of acute inflammation, which are a precursor to periodontitis.[6] Inflammation protects the host from potential infection by delivering antibacterial components from adjacent cells, restoring tissue function, and shielding the body from the detrimental effects of this damage.[5]

Gingival health is considerably affected by the inflammatory process.[5] Acute inflammation develops quickly and does not perpetuate, whereas chronic inflammation found in periodontal disease persists over an extended period of time.[5] Histologically, inflamed tissue contains large numbers of leukocytes, macrophages and lymphocytes,

which manifest as tissue necrosis and fibrosis, and result in both local and systemic damage.[5]

As a component of gingivitis diagnosis, microbial pathogens and gingival crevicular fluid (GCF) samples may also be analyzed.[4] Research has shown that inflammatory mediators in GCF including prostaglandin E2 and cytokines such as IL-1α and IL-1β have been associated with gingival disease.[4,5,48] Plaque biofilms emit several biologic components, which in turn generate micro-environments in which the organisms must adapt for survival.[5,17] The gram- positive and gram-negative bacteria in the biofilm subsequently colonize interproximally and near the gingival margin, resulting in pro-inflammatory byproducts such as protein toxins and endotoxins.[5] These byproducts permeate the epithelium and trigger the host immune response, which ultimately induce gingivits.[5] As the inflammation continues to progress, further mediators such as pro-inflammatory cytokines are created, which incite the movement of monocytes, T-cells, and neutrophils to the region.[5] Elevated serum levels of chemical mediators such as IL-1, IL-6, fibrinogen, and C-reactive protein are found in individuals with chronic gingivitis, as well as those with periodontitis.[5] Once active periodontal therapy is completed, clinical inflammation diminishes, and the levels of these chemical mediators decline.[5]

b. Microbes Affiliated with Natural Gingivitis

There are several components related to the progression from gingival health to gingival inflammation, including the presence and colonization of microorganisms, and the host response to this process.[18] When compared to samples taken from healthy subjects and those with periodontal disease, microbial plaque samples in subjects with

gingivitis differ in composition.[18] Microanalysis of oral plaque pathogens found in gingivitis demonstrates elevated quantities of microbes, including gram-negative organisms, filaments, and motile rods.[18] In a study conducted regarding the microbiology of gingivitis, Moore and colleagues discovered that a high preponderance of organisms found in periodontitis were present in smaller quantities in subjects with gingivitis.[49]

The microorganisms most commonly associated with gingivitis include those from the *Prevotella, Capnocytophaga, Streptococcus, Fusobacterium, and Actinomyces* species.[18,49] In addition, *Eikenella corrodens, Eubacterium nodatum, Campylobacter gracilis, Peptostreptococcus micros,* and *Campylobacter concisus* are also frequently found in subjects with gingivitis.[18,49] Certain organisms, including *Treponema* species*, Bacteroides forsythus, Campylobacter rectus, Veillonella parvula, Porphyromonas gingivalis, Prevotella intermedia,* and *Actinobacillus actinomycetemcomitans serotype a* are present in both gingivitis and periodontitis, with smaller quantities of organisms noted in subjects with gingivits.[18] Furthermore, the quantity of organisms found in periodontal disease is significantly higher than those found in periodontal health.[18]

c. History of the Experimental Gingivitis Model

The experimental gingivitis model was introduced in the 1960's by Dr. Harald Loe, Dr. Else Theilade, and Dr. S. Borglum Jensen.[50] Their groundbreaking clinical experiment to induce gingivitis in subjects with healthy gingiva through the absence of oral hygiene allowed them to analyze the succession of oral microbes and subsequent gingival inflammation.[50] Currently, the experimental gingivitis model is used in clinical

research to evaluate the resultant inflammatory response to the oral microflora as the process of moving from gingival health to gingivitis occurs.

For their first experimental gingivitis study, Loe and colleagues used twelve subjects who were either students or employees at their dental school.[50] The subjects were initially examined and scored with the Gingival and Plaque Index System, and were assessed for periodontal disease with the Periodontal Index System.[50] After the initial examination, the subjects were instructed to refrain from all oral hygiene practices for the duration of the study.[50] The subjects were reexamined with the same criteria at different time intervals based on the each individual's experimental period length .[50] Once a microbiological evaluation and a complete index was documented, and an inflammatory response was evident, the subjects were given explicit instructions on the use of wood and brush massage sticks to use for oral hygiene twice daily for rest of the experiment.[50] The examiner continued to evaluate the gingival and plaque scores during the oral hygiene phase, and the experiment concluded once the scores neared zero.[50] Microbial analysis of all subjects occurred in a range of six to ten intervals ranging from baseline until gingivitis was induced.[50] The final microbial analysis occurred once gingival health was restored.[50] The microbial types included gram positive cocci, spirochetes, filaments, short rods, fusobacteria, and vibrios.[50] In addition, the location and size of leukocyte existence was noted at each time interval.[50] The results of the study showed generalized plaque accumulation for all study subjects during the absence of oral hygiene, with a mean plaque index increase from 0.43 to 1.67, and a mean gingival index increase from 0. 27 to 1.05.[50] Once oral hygiene resumed, the scores decreased significantly.[50] The number of days for gingivitis to

clinically manifest varied; for three subjects, it took ten days, and the remaining nine subjects ranged from fifteen to twenty one days.[50] The gingival change assessment revealed increased inflammation interproximally when compared to the buccal gingiva, with the lingual gingival index demonstrating the lowest scores.[50] The interproximal site of maxillary molars exhibited the highest gingival index scores, and the lingual site of the mandibular premolars had the lowest scores.[50] One week after oral hygiene resumed, the entire group saw a marked decrease in inflammation and gingival index scores, from 1.05 to 0.11.[50] The initial microbial sample analysis revealed few microorganisms; however, once oral hygiene ceased, the microbial count expanded significantly, and was similar amongst all subjects with the exception of one.[50] There were three marked stages in microbial colonization during this experiment.[50] The initial phase was distinguished by large amounts of desquamated epithelial cells encompassed by coccal organisms, and a few leukocytes.[50] Approximately two to four days after oral hygiene ceased, the second phase began, and revealed large amounts of slender rods and filamentous shaped organisms in addition to the still abundant cocci.[50] In addition, the second phase also saw an increase in leukocyte aggregation.[50] It is noteworthy that one of the twelve subjects began the experiment with phase two microbial composition.[50] The microbial shift from phase two to three was not as discernible, as it progressed more slowly and at different points of time.[50] The transition to phase three typically occurred six to ten days after oral hygiene had ceased, and was marked by the addition of spirochetes and vibrios to the cocci, rods and filaments present in the flora.[50] Two of the subjects did not have visible spirochetes in their microscopic samples, but they displayed numerous vibros.[50] Leukocyte aggregation

was considerably abundant during phase three, and remained until the diagnosis of gingivitis was delegated.[50] Upon resumption of oral hygiene and return of gingival health, the final bacterial samples were taken, and ten of twelve subject's samples displayed a majority of short rods and cocci.[50] The other two samples showed an abundance of filamentous microbes, but vibrios or spirochetes were not observed in any of the twelve samples.[50] Although there were limitations to this study, the authors were able to establish the abundant increase and change in plaque microorganisms during the absence of mechanical plaque removal, and subsequent degree of change in gingival health.[50]

As a follow up to their initial experiment, Theilade and colleagues again embarked on another study, this time utilizing a longitudinal approach.[51] Initially, all of the subjects demonstrated excellent oral hygiene and gingival health, similar to the first study.[51] Once oral hygiene practices ceased, the plaque began to accumulate significantly, and the gingival index scores increased significantly as well, until they reached 1.0, which was considered mild gingivitis.[51] The amount of time it took to develop gingivitis within the group of subjects was relatively the same, and corresponded to the amount of plaque accumulation. Three subjects developed gingivitis in nine to thirteen days, five subjects developed gingivitis in fifteen days, and three subjects developed gingivitis in seventeen to twenty-one days.[51] The group that took the longest time to develop gingivitis also developed plaque accumulation slower than the other two groups. [51] However, for the time to reach a gingival index score of 1.0, the total plaque accumulation amongst the subjects was the same.[51] Additionally, the total plaque accumulation did not variate significantly between the maxillary and mandibular arches;

however, the interproximal areas exhibited the highest accumulation, while the oral surfaces (excluding the mandibular molars) displayed the lowest.[51] Incisor teeth showed the highest variability, with the interproximal areas showing heavy plaque accumulation, and the oral surfaces exhibiting significantly less amounts.[51] In regard to the gingival index scores, there was not as much variability as the plaque index scores, with the interproximal scores being the highest, and the oral areas of the incisors the lowest.[51] In addition, the facial and lingual scores of the maxillary molars were a bit lower than the corresponding mandibular molars.[51] Once oral hygiene was reinstated, the plaque and gingival index scores quickly returned to their original numbers, and the time to return to gingival health was roughly the same for all subjects.[51] Examination of the Periodontal Index System revealed no adverse effects of the gingival changes during the experimental period.[51] The change from a coccal flora to a filamentous flora took about 2 days (range one to four days) after oral hygiene ceased, and spirilla and spirochetes were observed after about seven days (range four to nine days) after oral hygiene ceased.[51] Visible characteristics of gingivitis clinically coincided with the development of the more complex flora in the areas.[51] However, it usually took seven or eight days from the time when total complex oral flora was developed in the area of the two maxillary left premolars until the participants had manifested generalized gingivitis.[51] When oral hygiene practices resumed, the change in the oral flora quickly returned to baseline levels.[51] Nine of eleven subjects' spirochetes and spirilla vanished within 24 hours of resuming tooth brushing, and in seven of eleven, the fusobacteria and leptotrichia were undetectable after 24 hours.[51] In ten of eleven cases, cocciform bacteria were the only ones visible once gingival health had returned.[51] Leukocytes

were undetected in the initial samples, but after four days of abstaining from hygiene, they were present in almost all samples, and continued to increase in amount and throughout the duration of the oral hygiene abstinence period.[51]

The purpose of this experimental gingivitis study was to attempt to establish a relationship between the bacterial oral flora of certain tooth surfaces, and the gingival condition of same tooth surfaces.[51] The results demonstrated that in these areas, the gingival index score corresponded to the oral flora in the plaque, and that slight gingivitis could be diagnosed at the approximate time the flora began to proliferate.[51] Furthermore, sub clinical inflammation was demonstrated earlier through the presence of leukocytes, and may be related to the initial phase of plaque formation.[51] The authors speculated their findings may implicate that the induction of gingivitis is related to the early changes in bacteria in the oral flora, expanding the findings of their initial study.[51]

There have been numerous other experimental gingivitis studies since these time-honored experiments, which have further substantiated the evidence between bacterial plaque accumulation and gingival health.[52] A study by Trombelli and colleagues utilized a randomized, split mouth localized experimental gingivitis trial to determine if and the extent to which the clinical parameters assessed during an experimental gingivitis model (such as plaque and gingival index scores) can be duplicated at separate times within certain populations (such as groups from previous studies who were identified as having different gingival inflammatory response to plaque accumulation).[53] Second, the consistency to develop a high or low gingival inflammatory response within these groups was evaluated.[53]

The results of this study reveal that a proportion of the subjects (50-59%), regardless of their initial classification of low or high responder, had a consistent high or low inflammatory response to the plaque accumulation during the repeat trial, and that this gingivitis model can be somewhat reproduced in selected populations when using well controlled, experimental conditions.[53] There was a statistically significant difference in regard to time and quadrant for the plaque index and cumulative plaque exposure.[53] The plaque index and cumulative plaque exposure scores in the test quadrants increased from day zero to day twenty-one.[53] In the control quadrants, the plaque index and cumulative plaque exposure scores remained similar to baseline, and were significantly different from the test-quadrant measurements at days seven, fourteen, and twenty-one.[53] In addition, statistically significant increases in the gingival index and the angulated bleeding score were observed in the test quadrants over the course of the trial.[53] The control quadrant gingival index and angulated bleeding score stayed close to zero throughout the trial, and were significantly different from the test parameters on days seven, fourteen, and twenty-one for both the angulated bleeding score and the gingival index.[53] When comparing the data from the two trials, no significant differences in the plaque index and angulated bleeding score were noted.[53] Gingival index scores were higher in the second trial, and were consistent in terms of the temporal changes throughout both trials in both groups.[53] Ten out of seventeen low responder subjects showed low susceptibility to inflammation after the second trial, and ten of twenty high responder subjects showed a high susceptibility to inflammation.[53] The results of this study reveal that the plaque and inflammation parameters during this experimental

gingivitis model are somewhat reproducible, and that a certain proportion of subjects are consistent with their response to plaque accumulation.[53]

A recent study by Lee et al used the experimental gingivitis model in a randomized, controlled, clinical trial of thirty subjects to ascertain how periodontal pathogens and biomarkers are modulated during bacterially induced gingival inflammation, and determine if they can use these results to coin individuals with a high response to gingivitis.[54] Mean gingival index, plaque index, and papillary bleeding score showed a significant increase during the experimental gingivitis induction, and decreased during the resolution when assessed at Day 35; however, no differences were observed in the IL-1 groups.[54] Participants were classified as either high or low responders depending on their inflammatory response, with a high gingival index >1.5 and a low gingival index < 1.5.[54] The baseline levels of salivary IL-6 and IL-8 revealed the highest capability to ascertain between the high and low responders.[54] Salivary biomarkers, MMP's, and bacterial biofilm were combined to develop receiver operating characteristic curves.[54] In this study, *Fusobacterium* species appeared to predispose subjects to an elevated inflammatory response, with high responders possessing a microbiological profile that could elevate colonization of periodontal pathogens.[54] The results of this study substantiate previous findings by demonstrating the use of saliva as an effective way to monitor critical gingivitis biomarkers, and that the combination of objective baseline parameters is very predictive of high responders related to the acute bacterial challenge during the experimental gingivitis model.[54] The microbial and clinical responses to the abstinence of oral hygiene were relatively consistent with previous experimental gingivitis studies.[54] This study also demonstrates that subjects with elevated baseline

levels of IL-6 and MMP-1 are at a higher risk of developing an elevated gingival inflammatory response when compared to subjects with low levels of these biomarkers.[54] Consequently, the results of this study could determine the microbiologic profile and biologic host response in the acute phase of gingival inflammation.[54]

d. Microbes Affiliated With the Experimental Gingivitis Model

A significant increase in the plaque accumulation during an experimental gingivitis model differs from traditional gingivitis, with the plaque accumulation demonstrating elevated quantities of *Actinomyces* organisms, which represents fifty percent or more of the microbes.[27] Furthermore, when compared to traditional gingivitis, experimental gingivitis plaques generally consist of gram-positive organisms consistent with those observed in subjects with healthy gingiva.[27]

Syed and Loesche performed a study to determine the effect of plaque age on microbes during an experimental gingivitis model.[55] The results demonstrated that succession of microbes is noted in oral plaque, and that *Actinomyces* were the predominant species throughout the three week study period.[55] Once the subjects reached the second and third week time interval, there was a significant increase in the both the relative amount and number of gram-positive rods, which occurred to the detriment of the gram-positive cocci.[55] The relative amount and number of the gram-negative species was consistent, but at the three week interval, the percentage of *Campylobacter (Vibrio) sputorum* isolates demonstrated a significant percentage of growth.[55] When compared to the quantity of *Streptococcus* and *Actinomyces* noted, the vibrios comprised a very small amount (1.2%) of the total organisms present.[55] The

most significant quantity of succession in the early (zero and one week) plaques was observed with *Streptococcus* organisms, and the *Actinomyces* organisms were predominant in the later plaques (two and three week), and increased not only absolutely, but also relatively, with *Actinomyces israelii* the most observed organism.[55] *Veillonella* species comprised fifteen to twenty percent of all colony forming units throughout the entire three week study period.[55] In addition, the amounts of other gram-negative organisms proliferated throughout the study, but at week three, they accounted for less than five percent of the total colony forming units.[55] These findings indicate that if total plaque accumulation is relative to gingivitis formation, then *Actinomyces* organisms had a significant role in the development of gingival inflammation in this experimental gingivitis study.[55]

2.6 Indices for Assessing Plaque and Gingival Inflammation

Indices to characterize gingival inflammation and plaque accumulation are often used in clinical research to provide parameters for clinicians to delineate the difference between health and disease.[56] The majority of gingival indices are comprised of minimum and maximum criteria scores on a ranked system, and utilize either bleeding, contour, color, crevicular fluid measures, or degree of involvement.[56] Numerical indices are relatively simple to use in epidemiological surveys and clinical trials, but are constrained by the lack of a ratio scale.[56] In addition, gingival indices are used to examine gingival appearance, which is not necessarily indicative of periodontal devastation.[56]

a. PMA Index

In the 1950's, the PMA index was developed to assess gingival health, and has been modified and used in clinical research repeatedly over the past few decades, with a particular benefit to pediatric research.[56] In this approach, the gingiva is divided into sections and is founded on the assertion that the interdental papilla (P) is the initiation site of inflammation, the inflammation then progresses to the marginal site (M), and in extreme circumstances, affects the attached gingiva (A).[56] Each area (P,M,A) is scored between zero and four, and it is hypothesized that the inflammatory location could be indicative of the degree of inflammation.[56]

b. Gingival and Plaque Index Systems

In the 1960's, renowned clinical oral health researchers Harald Löe and John Silness developed criteria for a gingival and plaque assessment, known as the Gingival Index System and Plaque Index System.[57,58] In their study of periodontal disease in pregnant and postpartum woman, these systems proved effective for evaluation of the degree of gingival inflammation and plaque accumulation.[57,58] The primary goal of developing these systems was to incorporate standards by which to evaluate gingival health relative to location, and provide uniform criteria by which to assess the location and degree of plaque accumulation.[56,59]

The clinical characteristics relative to the level of gingival inflammation were evaluated through four specific criteria, and are described as follows:

Criteria for the Gingival Index System [57]
0 = Absence of inflammation.
1 = Mild inflammation – slight change in color and little change in texture.
2 = Moderate inflammation – moderate glazing, redness, oedema, and hypertrophy. Bleeding on pressure.
3 = Severe inflammation – marked redness and hypertrophy. Tendency to spontaneous bleeding. Ulceration.

The clinical characteristics relative to the level of plaque accumulation were evaluated through four specific criteria, and are described as follows:

Plaque Index System Criteria[58]
0 = No plaque
1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2 = Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye.
3 = Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Prior to examination, either cotton rolls or a blast of air were used to dry the area.[57] One tooth from each sextant was selected for examination, including the maxillary right first molar and lateral incisor, the maxillary left first bicuspid, the mandibular left first molar and lateral incisor, and the mandibular right first bicuspid.[57] Four surfaces of each tooth (mesial, distal, buccal, and lingual) were given a score from 0-3, and the scores from the four surfaces were added together, and then divided by four, which yielded the gingival or plaque index score for the tooth.[57] The scores from individual

teeth could then be grouped together for a gingival or plaque index for that specific group of teeth.[57] Furthermore, the gingival index for the patient was assessed as an average of the surfaces evaluated, through adding the indices of the teeth and dividing by six.[57] The Gingival Index does not incorporate probing depth, clinical attachment level, bone loss, or any additional periodontal changes; the index is specific to gingival qualitative change.[59]

c. Retention Index System

In the late 1960's Löe modified these indices slightly, which included the use of entire dentition if preferred, and incorporated a new Retention Index System to evaluate the primary retentive measures through the quality of tooth surface near the gingival margin, which are described as follows:

Criteria for the Retention Index System[59]
0 = No caries, no calculus, no imperfect margin of dental restoration in a gingival location.
1 = Supragingival cavity, calculus, or imperfect margin of dental restoration.
2 = Subgingival cavity, calculus, or imperfect margin of dental restoration.
3 = Large cavity, abundance of calculus or grossly insufficient marginal fit of dental restoration in a supra- and/ or sub-gingival location.

The Retention Index System is similar to the Plaque and Gingival Index. All three of these indices provide reversible criteria for clinicians to utilize when screening subjects of all ages.[59] The adjustable nature of the criteria allows the clinician to select a specific number of teeth, or the entire dentition for evaluation.[59] These indices have proved to

be invaluable tools in clinical research, and modified versions of these indices are still in use today.[59]

d. Ramfjord Index / Russell Periodontal Index

Similar to the Löe and Silness indices, Ramfjord developed a periodontal disease index combined with a gingivitis index to assess gingival inflammation.[56] The gingiva of each tooth is scored between a zero and three.[56] In addition, Russell utilized a similar system with a range of zero to two.[56] Both Russell and Ramfjord's criteria do not specify different sites, and the results are subsequently more generalized than the approach used by Silness and Löe.[56]

e. O'Leary and Colleagues Gingival and Periodontal Index

The index developed by O'Leary and colleagues combines a gingival and periodontal index which separates the periodontium into two anterior and four posterior components.[56] The scores range from zero to three, with zero being slight to moderate inflammation that did not surround any single tooth, one being similar to one but entirely surrounding one or more teeth, two exhibiting notable inflammation, and three the presence of buccal or lingual recession.[56]

f. Suomi and Barbano Index

A index gauged on a three point scale (0-2) was created by Suomi and Barbano, and was comprised of assessing both lingual and facial surfaces of twelve areas of the periodontium including an anterior, premolar, and molar tooth from each quadrant.[56] To evaluate gingivitis, Fischman and colleagues developed an "end point" framework using the Löe Index, but measured gingival disease exclusively instead of

improvement.[56] This method was thought to be useful to ascertain effectiveness of therapeutic products in subjects currently diagnosed with gingivitis.[56]

g. Bleeding on Probing

An additional indice that is commonly used to assess gingival inflammation is bleeding on probing. Bleeding on probing presence is a reliable assessment of periodontal health that is easily evaluated clinically, and is a potential initial sign of gingivitis.[56,60]

In the 1970's, Ainamo and Bay developed a bleeding on probing index that was characterized by gentle probing of the gingival sulcus.[56] After a period of ten seconds, if bleeding was observed, it was recorded as positive, and the amount of positive sites were documented as a proportion of the total number of probed sites.[56] This method seemed feasible for both clinical dental practice and clinical research purposes.[56]

h. Sulcus Bleeding Index

In the 1950's, the Sulcus Bleeding Index was created, and bleeding on probing was the most prominent criteria.[56] The scoring criteria for this index is as follows:

Sulcus Bleeding Index[56]
0 = Healthy looking papillary and marginal gingiva, no bleeding on probing
1 = Healthy looking papillary and marginal gingiva, bleeding on probing
2 = Bleeding on probing and color change in gingiva
3 = Bleeding on probing, color change, slight edema
4 = Bleeding on probing, color change, obvious edema
5 = Spontaneous bleeding, color change, marked edema, ulceration

i. Papillary Bleeding Index

The Sulcus Bleeding Index was then modified and renamed the Papillary Bleeding Index, and was documented as the bleeding of gingiva after careful probing, and was classified as follows:

Papillary Bleeding Index[56]
0 = No bleeding
1 = Only one bleeding point present
2 = Several isolated bleeding points on a small area of blood
3 = Interdental triangle filled with blood
4 = Profuse bleeding spreading toward the marginal gingiva

j. Modified Papillary Bleeding Index

The Papillary Bleeding Index was then modified by Barnett and colleagues by depicting specific positioning of the periodontal probe using a light movement toward the mesial papilla from the mesial line angle of the tooth.[56] The appearance of bleeding was timed from the moment of probing, and was scored as follows:

Modified Papillary Bleeding Index[56]
0 = No bleeding within thirty seconds of probing
1 = Bleeding between three and thirty seconds of probing
2 = Bleeding within two seconds of probing
3 = Bleeding immediately upon probe placement

k. Edwards Bleeding Index

A bleeding index was created by Edwards in which dental tape was wrapped around a buccal or lingual proximal surface and placed into the base of the sulcus, then repeated twice.[56] Bleeding on probing was assessed using a dichotomous scale, with zero designated for absence of bleeding after fifteen seconds, and one if bleeding was

visible.[56] A similar approach was used by Loesche, and Caton and Polson, where a triangular shaped wooden wedge was used to generate interproximal gingival bleeding and assess the health of the papillae.[56]

2.7 Microbial Analysis

Traditional methods of microbial analysis have been limited by the narrow focal point on specific pathogens relative to a disease, and the capability to expedite the examination and identification of significant quantities of intricate microbes.[61] In the 1990's, alternative methods of examination were developed, which facilitated accelerated execution of subgingival plaque microbe classification, and further enhanced understanding of periodontal pathogens.[61] Furthermore, these methods allowed for the observation of the direct effect of therapeutic procedures on microbial plaque composition, and are useful in both diseased and healthy plaque samples.[61] In addition, these methods are also used in research to assess the relationship between microbial plaque and systemic disease.[61]

a. DNA Probes

Microbial subgingival plaque aggregation and the source of endodontic lesions have both been assessed by researchers with the use of whole genomic DNA probes, which are developed with the complete genome of a microbial classification as the goal.[61] Although this method is beneficial for the discovery of certain species, it is not free of limitations.[61] For example, the use of the complete genome yields the potential of cross-reactions amongst species, due to the mutual DNA shared between similar species.[61] In addition, it is possible that all types of a particular species may be undetectable by the probes, and result in a subsequent low sensitivity in regard to the

amount of cell detection.[61] However, these limitations have been disparaged to an extent by researchers at the Forsyth Institute, whose efforts have revealed these limitations can be refuted.[61]

Although the customary use of DNA probes has been successful for the establishment of specific microbes, very few are advantageous for assessing large numbers of microbial species.[61] On the other hand, regardless of direct or reverse procedure, checkerboard systems procure a significant increase in sample assessment for many species.[61] Because microbial plaque sample analysis has revealed the simultaneous occurrence of particular microbes, knowledge of the association between microbes could prove beneficial for disease control and prevention.[62]

b. "Checkerboard" DNA-DNA Hybridization

The "checkerboard" DNA-DNA hybridization method was initially described in 1994 by Socransky and colleagues.[61,63] In the past, techniques such as "reverse hybridization" were used, which assessed large quantities of target DNAs in relation to small samples.[63] The "checkerboard" technique was developed to utilize a sole support membrane to assess large quantities of DNA samples relative to large quantities of DNA probes, specifically with the aim of analysis of the vast bacterial complexes found in dental plaque samples.[63] This technique was modeled after previous use of the MiniBlotter™ and MiniSlot™ instruments during the preceding "checkerboard" method used for multiple antigen anti-body reactions assessed on a sole solid-support membrane.[63] In this study, the technique described previously by Smith and colleagues was used by Socransky and colleagues to isolate DNA from seven

references and thirty-six fresh isolates of *Campylobacter* and associated species.[63] This technique also allows the analysis of up to more than 100 experimental samples each day, creating further economic savings.[63] This "checkerboard" hybridization technique was used for the microbial plaque sample analysis in this experimental gingivitis study.

2.8 Colgate Total® Toothpaste

The emergent evidence indicating a relationship between oral and general health provides a need for safe, effective, clinically proven preventive oral health care products. In the 1985, the first dentifrice containing triclosan was introduced in Europe.[64] More than twenty years ago, the Colgate-Palmolive Company developed an exclusive toothpaste formulated with the well-known anti-microbial ingredient triclosan and a copoylmer.[8] In July 1997, the Food and Drug Administration (FDA) approved Colgate Total® for use as a preventive dentifrice for gingivitis, plaque and caries.[65] Colgate Total® contains 0.3% triclosan, which is a broad spectrum antimicrobial found in soaps, deodorants, and oral products, and 2% polyvinylmethyl ether maleic acid copolymer (PVM / MA), which increases the retention and uptake of triclosan to the buccal epithelium and enamel.[8] Use of this dentifrice can provide up to 12 hours of antimicrobial protection and subsequent control of biofilm.[8] Currently, Colgate Total® is the only dentifrice with triclosan available in the United States.[8] Triclosan containing toothpaste and mouth rinses without the copolymer formulation are available for use in other countries.[8]

2.9 Triclosan

Triclosan is a broad spectrum, synthetic antimicrobial compound that has been safely used for almost 40 years in deodorants and soaps with no noted adverse events.[8,66] In 1969, triclosan was registered for use as a pesticide, and is used in this capacity for industrial, commercial, and institutional purposes.[66] Triclosan is also used as a preservative in many materials including plastics, fabrics, and adhesives.[66] The chemical structure of Triclosan (2´-hydroxy-2,4,4´-tricholrodiphenyl ether) fundamentally resembles thyroid hormones.[66,67] Triclosan is considered to be non-toxic in mammals, and research studies of up to four years of triclosan containing dentifrice use revealed no change in the hematological, clinical, or biochemical measurements assessed in the subjects.[67] However, concentrations of triclosan relative to the use of consumer products with triclosan have been detected in urine, breast milk, and human plasma.[67] As a result, researchers evaluated the effect of 14 days of triclosan dentifrice use on thyroid hormone levels found in plasma to determine if there was a significant effect.[67] Although there was no notable difference in thyroid hormone levels, the plasma concentration of triclosan was higher than what was described previously.[67]

The Environmental Protection Agency (EPA) reregistered triclosan for pesticide use in 2008 based on the results of animal studies, and a portion of the urinary concentration data obtained from the 2003-2004 NHANES survey.[66] A human health risk assessment was performed to analyze available animal study data on the residue and product chemistry, residential and occupational exposure, toxicology, and additional readily obtained literature to substantiate the reregistration.[66] Evidence in regard to carcinogenicity, reproductive and developmental toxicity, endocrine effects, and chronic

toxicity of triclosan was evaluated.[66] Because the 2003-2004 NHANES data was also utilized, the 2008 assessment is regarded to be inclusive of regulatory uses of both EPA and FDA exposures of triclosan.[66] The EPA is in the process of updating this assessment based on the urinary results of the 2005-2006 NHANES data, and will revise this assessment if deemed scientifically necessary.[66] In addition, recent animal study data on the effect of triclosan on estrogen and thyroid hormones has prompted further research on the potential risk of triclosan exposure and use in humans.[66] In 2013, the EPA will continue to monitor endocrine research, and conduct an additional widespread assessment of triclosan, and will modify their regulatory decision if warranted.[66] Furthermore, the EPA will maintain involvement in the Interagency Task Force on Antimicrobial Resistance, collaborate with the FDA to coordinate data on triclosan, and confer requirements for additional research studies that will benefit both the FDA and EPA in determining if triclosan is safe for human use.[66]

Several studies have confirmed the safety and lack of microbial resistance due to long-term triclosan dentifrice use.[8,67,68] The 1986 American Dental Association Guidelines for Acceptance of Chemotherapeutic Products for the Control of Supragingival Dental Plaque and Gingivitis contains a stipulation pertaining to microbiological monitoring.[69] In order to fulfill this stipulation, monitoring of the oral microflora was incorporated into four long-term clinical efficacy studies on plaque and gingivitis, and each of these studies substantiated that long-term use of triclosan copolymer dentifrice did not induce changes in the microbial composition of supragingival plaque to favor development of pathogenic, opportunistic, or resistant microorganisms.[69] The lack of adverse effects in these studies were further

substantiated by six additional long-term studies that were constructed solely to assess the effect of triclosan copolymer dentifrice on the oral microbes reviewed in these studies.[69]

Cullinan et al conducted a sub-study of 132 subjects from their Cardiovascular and Periodontal Study, which was investigating the effect of 0.3% triclosan toothpaste on the progression of chronic periodontal disease in subjects with cardiovascular disease.[67] This sub- study was conducted to specifically evaluate the use of triclosan dentifrice on thyroid function over a four year period.[67] Paired serum samples from year one and five of the levels of thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), antithyroglobulin antibody (anti-TGab), and antithyroid peroxidase antibody (TPOab) were assessed.[67] At the conclusion of the four year study, there was no significant noted effect of triclosan toothpaste use on thyroid function.[67] The results support the opinion that 0.3% triclosan in toothpaste is safe for long term use, without the risk of adverse effect on thyroid function.[67] In addition, the subjects in the triclosan group had been using the toothpaste for a year before the first serum samples were taken, and the results were similar in both groups, yielding evidence that the triclosan dentifrice had no effect on thyroid function during the intervening time period.[67]

Cullinan et al conducted an additional sub-study of 40 subjects from their Cardiovascular and Periodontal study to determine triclosan resistance in oral plaque samples after a long-term period of triclosan dentifrice use (five years).[68] This study revealed that using a concentration of 0.3% triclosan, there was no growth of bacteria in either group during anaerobic or microaerophilic conditions.[68] Lower triclosan concentrations showed similar growth in both groups, and the vast majority of

identifiable bacteria were common among both of the groups.[68] In addition, the triclosan and minimum inhibitory concentrations of bacterial isolates in both groups were similar, revealing that use of triclosan dentifrice over the five year period did not yield an increase in the minimum inhibitory concentration from dental plaque.[68] The results of this study have provided evidence that triclosan dentifrice use does not lead to oral plaque bacterial resistance over a long period of time.[68]

2.10 Polyvinylmethyl Ether Maleic Acid Copolymer

PVM/MA copolymer and its related salts and esters are used primarily as binders in a variety of products, including soaps, deodorants, oral health products (including Colgate Total®), cosmetics, hair care products, and film formers.[70] A 1993 safety assessment of ethyl and butyl ester of PVM/MA copolymer was conducted by the Cosmetic Ingredient Review Expert Panel, and this data revealed evidence of the safety of the salts and esters of the following PVM/MA copolymers: calcium/sodium, potassium ethyl ester, sodium ethyl ester, potassium butyl ester, sodium butyl ester, and isopropyl ester.[70]

PVM/MA copolymer is included in the Code of Federal Regulations for its use in dental products, with calcium/sodium PVM/MA copolymer for its use in dental adhesives.[70] In addition, PVM/MA copolymer and butyl ester are listed for use in dental paste and a topical solution at 30% on the Food and Drug Administration inactive database.[70] Several published clinical studies on the efficacy of toothpaste containing PVM/MA copolymer were evaluated, and no adverse effects were noted from PVM/MA copolymer.[70] Although there is a lack of data on the reproductive and developmental toxicity and the carcinogenicity of PVM/MA copolymer, due to the large molecular

weight structure, it was concluded that the skin would not readily absorb PVM/MA.[70] In addition, there was no information located on the distribution, elimination, metabolism, absorption, or general biology of PVM/MA and its related salts and esters in the literature.[70] The panel concluded that PVM/MA copolymer and its related salts and esters are safe to use in cosmetic products.[70]

2.11 Clinical Efficacy of Colgate Total® Toothpaste

The outcomes of several studies have demonstrated the reduction in gingivitis and subsequent benefit to periodontal health due to the anti-inflammatory and antimicrobial action of Colgate Total® toothpaste with triclosan copolymer when compared to placebo.[8] These results convey that Colgate Total® may be used as an evidence-based recommendation to improve and sustain periodontal status, and inhibit further disease devastation.[8]

There have been several studies and systematic reviews on the clinical efficacy of triclosan containing toothpaste (with and without copolymer) on plaque control and gingivitis.[9–12] Davies an colleagues conducted a systematic review of 16 studies comparing Colgate Total® to a fluoride dentifrice for their effect on plaque and gingivitis.[10] In 13 of the 16 studies, the subjects received a prophylaxis prior to the beginning of the studies, which reveals the capability of Colgate Total® to control new plaque formation.[10] In the three studies without initial prophylaxis, there was indication of the effectiveness of existing plaque removal of the two study toothpastes.[10] The more prominent clinical significance revealed through the meta-analysis was the reduction in gingival bleeding and overall improvement in gingival health.[10] The results

of this systematic review suggest that unsupervised use of a triclosan /copolymer containing dentifrice (Colgate Total®) showed a significant improvement in supragingival plaque removal and improvement of gingival health when compared to a fluoride dentifrice.[10]

A recent study by Fine et al evaluated the antimicrobial effects of triclosan containing toothpaste compared with two other dentifrices on *Streptococci*, *Actinomyces*, hydrogen-sulphide (H(2) S)-producing bacteria, *Fusobacteria*, anaerobes, and *Veillonella* samples on four oral sites.[71] The results of this study showed that the subjects using Colgate Total® had statistically significant reductions at all sites and with all evaluated organisms, when compared to the two other test dentifrices, up to twelve hours post brushing.[71] In addition, intergroup comparisons also revealed greater efficacy of Colgate Total® when compared to the SnF_2/SHMP or NaF groups.[71]

An additional crossover study by Fine and colleagues evaluated the *in vivo* effect of triclosan/copolymer toothpaste compared with a control fluoride toothpaste, and included *Veillonella* species, *Fusobacteria* species, total cultivable anaerobes and hydrogen sulfide (H(2)S)-producing bacteria.[72] The results demonstrated that the subjects brushing with the triclosan-copolymer toothpaste has statistically significant reductions (90 percent or higher) in both tongue and plaque anaerobic microflora, and an 88-89 percent reduction in salivary anaerobic bacteria at both the 6 and12 hour time points, when compared to the control dentifrice.[72] The results of the *Veillonella*, *Fusobacteria*, and H_2S producing oral bacteria also showed statically significant reductions at both time points when compared to the control dentifrice.[72] The results of

this study show the clinical efficacy of triclosan-copolymer toothpaste on oral microorganisms for up to twelve hours.[72]

A randomized, controlled, clinical trial was conducted by Ozaki et al to determine the efficacy of a herbal dentifrice (Parodontax®) in the reduction of plaque and gingivitis.[73] The researchers used Colgate Total® toothpaste as the control, and tested the dentifrices on subjects with established gingivitis, which provides evidence of the true effect of an antimicrobial ingredient on gingivitis.[73] The results showed both toothpastes were significantly effective at reducing plaque and gingival index scores over a 28 day period.[73] At baseline, there was no significant difference between the plaque scores and GI of the two groups.[73] At the day 28 evaluation, the treatment group showed a 19.9% reduction in buccal and lingual plaque, and the control group demonstrated an 18.3% reduction.[73] In regard to the plaque on the proximal surfaces, the treatment group showed a 19.3 percent reduction, and the control group 15.4 percent redcution.[73] Although both groups showed a significant decrease in plaque score, the difference was not statistically significant between the groups. The day 28 gingival index scores showed a mean reduction of 28.4 percent in the buccal and lingual surfaces in the treatment group, and 36.3 percent reduction in the control group.[73] In regard to the proximal surfaces, the test group showed a 23.5 percent reduction, and the control group 32.5 percent. Although both groups showed a significant decrease in the gingival index, there was no statistical difference between the groups.[73] In addition, no adverse events were reported among either group.[73] Furthermore, the results revealed that the Parodontax® herbal dentifrice was as effective as Colgate Total® at reducing plaque and gingivitis.[73]

2.12 Triclosan Dentifrice /Mouthrinse Formulations Available in Other Countries

There are several triclosan dentifrice / mouthrinse formulations available for consumer use in other countries. A study was carried out by Prasanth to determine the effect of several toothpastes, including triclosan containing toothpaste, on *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans*.[64] The results of this study show that the triclosan containing dentifrice (toothpaste A) had the highest zones of inhibition against *E. coli* ($p < 0.001$) when compared to all of the other toothpaste formations.[64] For *S. mutans* and *C. albicans*, the zones of inhibition were less than *E. coli*, but were significantly different at higher dilutions (1:8, 1:16, $p < 0.05$) for the triclosan containing dentifrice.[64] In addition, all of the mouthrinses (mouthrinse formulation F and J contain triclosan) tested showed significant differences against *E. coli*, but Chlorohexidine Gluconate, Sodium Fluoride, and Zinc Chloride mouthrinse had the greatest significance.[64] In addition, mouthrinses F, G, and J showed significant differences when compared to formulations H and I for their effect on *S. mutans*.[64] The effect on *C. albicans* showed the zones of inhibition were significant for mouthrinse F.[64]

A clinical trial was performed by Pradeep and colleagues to assess the effect on *Streptococcus* and *Actinomyces* species with the use of triclosan co-polymer and amine containing dentifrices.[74] After 24 weeks, the results of this study demonstrated the clinical efficacy of both the triclosan-copolymer and amine fluoride dentifrices when compared to the placebo dentifrice.[74]

Otten et al conducted an unblinded, clinical substantivity study of 74 subjects to analyze the effect of plaque and saliva on the prolonged substantivity of three

antibacterial dentifrices when compared to a non-antibacterial control dentifrice, and to compare the potential changes of bacterial composition of saliva and plaque post brushing with different dentifrices.[75] The authors hypothesized that plaque remaining post-brushing could function as a reservoir for antibacterial dentifrice components, and increase their substantivity (continual therapeutic benefits although brushing has ceased).[75] Although both Colgate Total® and Crest Pro-Health® decreased bacterial viability in plaque samples up to 12 hours post brushing, only remaining plaque from the subjects who brushed with Crest Pro-Health® had significant residual antibacterial activity 12 hours post brushing that could contribute to killing bacteria in unexposed plaque over a long period of time.[75] Additionally, there was a lack of statically significant evidence of the direct effect of bacterial viability in saliva across all samples.[75] Overall, plaques samples obtained post use of both the Crest and Colgate dentifrices demonstrated lower viabilities than the control plaques, while the use of Zendium showed similar viability when compared to the control plaque.[75]

A crossover *in vivo* study by Sreenivasan et al was designed to evaluate early dental plaque formation in the human oral cavity, and to assess the effects of dietary and oral hygiene practices on the formation of biofilm.[76] A custom butterfly device made from dental acrylic was constructed from mandibular impressions of the subjects.[76] After a one week “washout” period, the subjects placed the device in their mouth, with the goal of microbial accumulation in mind.[76] Several treatments, including mouthrinses and dentifrices, were tested for their effects on the microbial colonization on the device.[76] The results showed that large amounts of oral microbes colonized the device by two hours, and they increased significantly by four hours.[76] Colonization of bacteria

increased significantly after rinsing with the 10 % sucrose solution, but remained unaltered after rinsing with potable water, the fluoride rinse without antimicrobial properties, or brushing with the fluoride toothpaste.[76] However, rinsing with the chlorohexidine gluconate, cetylpyridinium chloride, or triclosan copolymer mouthrinse demonstrated significant colonization inhibition, with a dose dependent inhibition noted with the chlorohexidine rinses.[76] In addition, brushing with Colgate Total® also significantly inhibited colonization of the microbes when compared to the control dentifrice.[76]

2.13 Study Objectives and Overview

The purpose of this pilot study was to determine the effect of Colgate Total® Clean Mint toothpaste on microbial pathogens during an experimental gingivitis model in the total absence of mechanical interruption of plaque biofilm. Previous studies have focused on the effects of triclosan containing dentifrices on oral microflora; however, few studies have examined the effects of triclosan dentifrice on microbial pathogens in the total absence of mechanical interruption of plaque biofilm. If the biofilm is not removed through the mechanical effects of brushing, flossing, or other oral hygiene aids, the biologically active by-products released from the biofilm infiltrate the gingival epithelium, inducing gingivitis.[5] The intent of this research was to study the effects of Colgate Total® toothpaste in the absence of mechanical plaque biofilm removal during an experimental gingivitis model. The elimination of mechanical plaque removal determined the sole effect of Colgate Total® toothpaste on microbial pathogens and gingival health.

Potential outcomes of this study could prove to be invaluable for dental hygienists. The foundation of dental hygiene is prevention, and a dentifrice known to inhibit microbial plaque proliferation and improve gingival health could be an essential component of homecare product recommendation. The goal of this study was to determine if Colgate Total® toothpaste is effective at reducing microbial pathogens that are prevalent in plaque biofilm samples. Lack of proper oral hygiene is common among patients, and insufficient plaque control increases the potential for gingivitis to progress into periodontitis. The emerging relationship between periodontal disease and systemic disease validates the need for further research on the preventive capability of Colgate Total® toothpaste. The results of this study are beneficial for dentifrice recommendations in clinical dental hygiene practice, and may potentially improve patient's oral and general health.

a. Specific Aim / Hypothesis

Specific Aim:

The specific aim of this study is to determine the effect of Colgate Total® toothpaste on microbial pathogens in plaque biofilm samples during an experimental gingivitis model in the absence of mechanical interruption of plaque biofilm.

Hypothesis: Colgate Total® toothpaste, as compared to a standard of care toothpaste, will be more effective in reducing microbial pathogens in plaque biofilm samples during the experimental gingivitis model.

CHAPTER III

MATERIALS AND METHODS

This research study was conducted during a randomized, controlled, clinical trial at the University of Michigan Center for Oral Health Research (MCOHR). This was a pilot study, with the goal of acquiring data to support a future study on a larger scale. The total number of subjects enrolled for participation was thirty, with fifteen subjects in each arm of the study.

3.1 Examiner Calibration

Prior to enrollment of study subjects, all study examiners were required to participate in a training and calibration exercise for clinical parameters, intra-oral photos, and sample collection at MCOHR.

3.2 Clinical Periodontal Measurements

The clinical periodontal measurements were assessed using a Hu-Friedy (Chicago) North Carolina Probe. Six measurements were recorded on all teeth (except third molars), rounding down to the nearest millimeter. Assessed periodontal parameters included clinical attachment level (CAL), free gingival margin (FGM), and probing pocket

depth (PPD). The calculation of total clinical attachment level was from the CEJ or an additional fixed reference point to the base of the sulcus. Bleeding on probing (BOP) was assessed using a dichotomous scale, with “0” indicated absence of BOP, and “1” indicating the presence of BOP.

3.3 Sample Collection

All samples were collected in accordance with the study protocol.

3.4 Saliva Collection

Unstimulated whole saliva was obtained at the beginning of each study appointment, and stored at -80° Celsius until it was ready for analysis as described previously.[33,77,78] To remove gross debris, the subjects were informed to rinse their mouth vigorously with water for 20 seconds, then expectorate. After a two minute waiting period to establish baseline levels of saliva, the subjects tipped their head toward the graduated test tube and expectorated whole saliva in the plastic funnel inserted in the plastic, sterile tubes, labeled with the subject’s initials, sample name, and date of harvest. The subject had a maximum of 15 minutes to expectorate 2 ml of whole saliva. Following completion of sample collection, the sample was immediately placed on ice, aliquoted, and supplemented with a proteinase inhibitor combination of 1% aprotinin (1mg/ml) and 0.5% phenylmethylsulphonyl fluoride (PMSF) (200mM in MeOH) (Sigma Chemical Company, St-Louis, MO) prior to storage at -80°C.

3.5 Gingival Index

Gingival Index was determined as previously described by Loe and Sillness.[57]

TABLE 1
Gingival Index

Scores	Criteria	Bleeding
0	Absence of inflammation	No bleeding on probing
1	Mild inflammation Slight change in color and texture	No bleeding on probing
2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

3.6 Plaque Index

Plaque Index was determined as previously described by Silness & Loe.[58]

TABLE 2
Plaque Index

Scores	Criteria
0	No plaque
1	A film of plaque adhering to free gingival margin and adjacent area of tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

3.7 Gingival Crevicular Fluid Sampling

Gingival crevicular fluid samples were collected and analyzed as previously defined by Lampster and Ahlo (77) and Giannobile et al (78). [79,80] GCF samples were obtained from the mesiobuccal or distobuccal site of two mandibular teeth assigned through randomization. The sample did not contain plaque, blood, or saliva. If supragingival plaque was visible, it was removed gently with a curette prior to sampling. Cotton roll isolation was used around the sample site, and then the site was dried with gauze and a small amount of air from the air/water syringe. Care was taken to not direct any air flow into the gingival sulcus. Forceps were used to insert the white cellulose portion of the methylcellulose strip (Periopaper®, Pro flow, Inc. Amityville, NY) into the sulcus until a gentle resistance was felt, similar to periodontal probing. The strip remained in position for 30 seconds until it was removed. Care was taken to ensure the area remained isolated. If a site required re-sampling, a minimum of 90 seconds elapsed before resampling occurred. After the GCF sample collection was completed, each strip was inserted into a microfuge tube and labeled with the subject's initials, study number, date, and tooth location. The tube was then placed onto dry ice for transportation to the laboratory, and is then stored in a -80° Celsius freezer until analysis. Because the strips were not stored with protease inhibitors or stabilizers, it was recommended that subsequent analysis be conducted as soon as possible to minimize protein degradation.

3.8 Gingival Crevicular Fluid Analysis

Harvested crevicular fluid proteins were extracted from the GCF strips utilizing an elution method adapted from Giannobile et al.[80] This process involves a series of

washes and centrifugations. To inhibit protease activity, the elution buffer used during the GCF protein extraction was made fresh and kept on wet ice during the entire extraction process. The ingredients in the GCF buffer include 24.5mL Phosphate Buffered Saline, pH 7.4, 125 µl phenylmethylsulphonyl fluoride (PMSF; Sigma Chemical, St. Louis, MO); 200mM in MeOH, 250 µl Aprotinin (Sigma Chemical, St. Louis, MO); 1 mg/ml in water, and 83.5 µl of 30% Human Serum Albumin (Sigma Chemical, St. Louis, MO). The GCF strips were placed on wet ice from the -80° Celsius freezer, and kept there for the duration of the procedure. 20 µl of the extraction buffer was then pipetted onto the white (cellulose) area of the GCF strips. The strip was then secured at the top of a 12 X 75 ml polypropylene tube labeled with the subject's initials and study number, tooth location, and harvest date. A cap was used to keep the orange area of the strip in the correct place. The tubes were then centrifuged at 2000 rpm at 4 degrees Celsius for 5 minutes. This centrifugation process was repeated an additional four times until a total volume of 100 µl was achieved. The entire product (100 µl) was then placed into a sterile microfuge tube, and stored at -80° Celsius until used.

3.9 Plaque Biofilm Collection

To obtain supra and subgingival plaque samples, a sterile Gracey 11/12 or 13/14 curette instrument was used. Samples were collected from the mesiobuccal or distobuccal surface of the allocated study appointment teeth, and stored at -20° Celsius until processing occurs. A different tooth was selected for sampling at each visit. This allowed the quantitative and qualitative changes from plaque maturity to be noted. The tooth was dried with air, and then the Gracey curette was inserted into the sulcus until resistance was felt. A stroke with light pressure similar to scaling was used to remove

the plaque sample. Immediately after obtaining the sample, it was placed into a vial labeled with the subject's initials, study number and sample type, and harvest date, that contained 150 µl of TE (10mM Tris-HCl, 1 mM EDTA, 500 ml Distilled Water, pH 7.6). The curette was then rotated for 5 seconds to immerse the plaque bacteria sample into the solution. 100 µl of 0.5 M NaOH was added into each vial, and the samples were stored at -20° Celsius until they were processed.

3.10 Biomarker and Microbial Analysis

Ten biomarkers were analyzed in the gingival crevicular fluid and saliva samples, including IL-1α, IL-1β, IL-6, IL-8, IL-10, MCP-1, MMP-8, MMP-9, TIMP-1, and TIMP-2. Forty bacterial microbes were analyzed using the checkerboard DNA-DNA hybridization technique as previously described by Socransky et al, including *Streptococcus mutans, Streptococcus gordonii, Actinomyces viscosus, Veillonella parvula, Campylobacter rectus, Prevotella intermedia, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola and Candida albicans.*[63] The microbial analysis was conducted at Dr. Ricardo Teles' Lab at the Forsyth Institute in Boston, Massachusetts.

3.11 "Checkerboard" DNA-DNA Hybridization

The samples were first boiled for a period of 5 minutes. Once cooled, 800 µl of 5M ammonium acetate was placed in each vial. The contents of the vials were subsequently pipetted into the Miniblot apparatus. Next, DNA was isolated from the individual bacteria and then quantified with a spectrophotometer. Each DNA sample was adjusted to 1 ng/ml and loaded inside the channels of the Miniblotter 45 and placed onto Hybond-N+ nylon membrane. The nylon membranes were then incubated at 4°

Celsius overnight. Next, the membranes were removed from the device, and the DNA was denatured and fixed in a solution of 0.4M NaOH and 1.5M NaCl. The membranes were then rinsed in 2X SSC (1x = 0.15M NaCl, 0.015M sodium citrate, pH 7.0). The membranes were pre-hybridized at 42° Celsius for 1 hour in 50ml of a solution consisting of 50% formamide, 5x SSC, 1% casein, 5x Denhardt's solution (1x = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 25mM sodium phosphate (pH 6.5), and 0.5 mg/ml denatured salmon sperm DNA. Then, the membranes were placed in the Miniblotter 45, and rotated 90 degrees from the original location. Digoxigenin-labeled whole chromosomal DNA probes were made from the individual test strains utilizing the random primer method. The probes and hybridization buffer were mixed and injected into separate lanes of the Miniblotter. Saran Wrap was used to wrap the entire apparatus and it was stored in a Ziploc bag to prevent evaporation. The membranes were then hybridized by gentle shaking overnight at 42° Celsius using a hybridizing solution containing 20ng/ml of labeled probe, 45% formamide, 5x SSC, 1x Denhardt's solution, 20mM sodium phosphate (pH 6.5), 0.2mg/ml denatured herring sperm DNA, 10% dextran sulfate, and 1% casein. The membranes were then washed twice at low stringency (25° Celsius in 200ml 2x SSC, 0.1% SDS for 5 min.), and then twice at high stringency (65° Celsius in 6L of 0.1x SSC, 0.1% SDS for20 min). For hybrid detection, membranes were blocked with 1% casein in maleate buffer (100mM maleic acid, 150mM NaCl, and pH 7.5). Next, they were incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase, and diluted with a 1:20,000 in maleic buffer. Once washing was completed, the membranes were incubated for 1 hour at 37° Celsius in 4-methoxy-4-(3-phosphatephenyl)-spiro

(1,2-dioxetane-3,2'-adamantane) disodium salt, and exposed to x-ray film for time periods ranging from 1-30 min.

3.12 Timeline of Study Procedures

SCREENING PHASE	EXPERIMENTAL GINGIVITIS PHASE				RECOVERY PHASE
Day -14	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 35**
Informed consent Medical History Clinical Measures Urinary Analysis Prophylaxis OHI Impression For Stent	Medical History Updated Concomitant Medications Noted Report of Adverse Events Radomization of Study Arm and Stent Side Intra-oral Photos Plaque Sample Collection Saliva Samples BOP Assessment Gingival and Plaque Index GCF Samples Instructions	Follow Up Phone Call/ Email	Medical History Updated Concomitant Medications Noted Report of Adverse Events Intra-oral Photos Plaque Sample Collection Gingival and Plaque Index *GCF Samples Saliva Samples Instructions Incentive Payment *samples will be collected after abstaining from tooth brushing for10-12 hours and at 1,2,4, and 6 hours after brushing with stent in place	Medical History Updated Concomitant Medications Noted Report of Adverse Events Intra-oral Photos Plaque Sample Collection Gingival and Plaque Index *GCF Samples Saliva Samples Prophylaxis Collection of Stents & Unused Study Toothpaste Incentive Payment	Medical History Updated Concomitant Medications Noted Report of Adverse Events Intra-oral Photos Saliva Samples Gingival and Plaque Index Plaque Sample Collection GCF Samples Incentive Payment

3.13 Screening Phase: Day -14 Study Appointment

Informed consent was read, comprehended, and signed by the subject (see Appendix B). A comprehensive medical history was obtained. Clinical measures including an oral and periodontal exam were performed, including probing pocket depth, recession, clinical attachment level, and bleeding on probing (see Appendix H). If the subject met the clinical eligibility requirements for study participation, urinary analysis was conducted of cotinine levels to determine smoking status. The subjects were given a prophylaxis, including polishing and oral hygiene instructions. Colgate Regular Flavor® toothpaste and Colgate floss was dispensed to each subject. Subjects were instructed to not rinse with anti-microbial mouth rinses or brush with anti-inflammatory toothpastes. An impression of the subject's mandibular arch was taken for stent fabrication. A thin layer of silicone material was applied to block out undercuts on the

poured model. In addition, a 1mm thick layer of silicone that extends from 3mm below the free gingival margin up to the cervical half of the teeth was applied to provide sufficient space for the allocated toothpaste to remain in the appropriate site. The purpose was to minimize loss of allocated toothpaste out of the stent while ensuring the maximum amount stayed accumulated between the stent, teeth, and gingiva. A thermoelastic material comparable to Durasoft (3mm thick), was used for stent fabrication. The material was heated and vacuum formed to the prepared study models. The fabricated stent covered one-half of the mandibular arch, and was trimmed until it extended 1mm short of the mucobuccal and mucolingual folds in the buccal and lingual vestibule.

3.14 Experimental Gingivitis Phase – Day 0 Study Appointment (Baseline)

The subjects returned fourteen days after the initial screening visit to evaluate their BOP score (see Appendix I). The medical history and accompanying medications were reviewed and updated if necessary, and reported adverse events were documented. Subjects presenting with a BOP score $\leq$ to 10 percent began the experimental gingivitis phase at this appointment. Those with a BOP $\geq$ 10 percent received further oral hygiene instructions, and were requested to return for an additional appointment in two weeks. Subjects who failed to achieve a BOP score $\leq$ 10 percent at the second appointment were deemed ineligible to participate in the study.

Eligible subjects were randomized into an arm of the study. The assignment of study arm (treatment or control) and mandibular stent (right or left) was done through randomization (see Appendix E). The study coordinators had sole access to the

randomization chart. The examiners and subjects were both blinded to the assignment of study arm. The treatment group was allocated Colgate Total Clean Mint® toothpaste. The active ingredients of this dentifrice are sodium fluoride 1100 ppm 0.243% (0.14% w/v fluoride ion), and triclosan 0.3%.[81] The inactive ingredients are water, hydrated silica, glycerin, sorbitol, PVM/MA copolymer, sodium lauryl sulfate, cellulose gum, flavor, sodium hydroxide, propylene glycol, carrageenan, sodium saccharin, mica, titanium dioxide, and FD&C Blue 1.[81] The control group was allocated Colgate Great Regular Flavor® with 1000 ppm MPF. The active ingredient of this dentifrice per tube is sodium monofluorophosphate 0.76% (0.15% w/v fluoride ion).[82] The inactive ingredients per tube include dicalcium phosphate dihydrate, water, glycerin, sorbitol, sodium lauryl sulfate, cellulose gum, flavor, tetrapotassium pyrophosphate, and sodium saccharin.[82] The same style of toothbrush was dispensed to all study participants, and they were informed to cease usage of all oral home care products except for those received as part of the study. To ensure the absence of all mechanical plaque removal in the stent area, subjects were instructed to refrain from flossing their entire dentition for the duration of the study.

Each subject had six intra-oral photos taken of the mandibular arch. Collection of whole saliva occurred as previously described by Mandel.[78] The Plaque Index (PI) and Gingival Index (GI) of the mandibular arch was assessed. Gingival crevicular fluid samples (GCF) were acquired from the mesiobuccal or distobuccal surface of two mandibular teeth. Saliva and GCF samples were taken at Day 0, 14, 21, and 35 of the study appointments. Determination of the two sites (teeth and surface) that were selected at the study visits to collect the plaque and GCF samples was done using a

randomization chart (see Appendix E). If the subject was missing a posterior tooth, the canine was chosen in replacement. Implants were excluded from selection for plaque and GCF samples. Repeat GCF samples were collected on Day 14 and 21 of the study.

The subjects received specific home care instructions (see Appendices J and K). Each subject was advised to brush their teeth with their allocated study toothpaste twice daily for two minutes. Prior to brushing, the subjects were instructed to place 2ml / 2.6 g of their allocated toothpaste into the stent, and then place the stent in the assigned area. The toothpaste was filled into a 6ml plastic syringe by placing the open toothpaste tube against the barrel of the syringe, and pulling with gentle pressure to fill the syringe to the 2ml demarcation.[83] The subjects then evenly distributed this quantity of toothpaste into the indentations in the stent.[83] The stent remained in place until the two minutes of brushing was completed. The stent was then removed, rinsed with water, and stored in an appliance case until the next brushing period.

3.15 Experimental Gingivitis Phase - Day 7 Study E-mail or Phone Call

Each subject was contacted by one of the research team members to ascertain their compliance of the study protocol and determine if they had any concerns that need to be addressed (see Appendix D). The research team member reaffirmed the study protocol for stent use and discontinuance of oral hygiene. If any concerns were noted, a follow up appointment was scheduled with the principal investigator of the study and the subject.

3.16 Experimental Gingivitis Phase – Day 14 Study Appointment

Prior to this appointment, the subjects were informed to abstain from brushing their teeth for 10-12 hours. The medical history and accompanying medications were reviewed and updated if necessary, and reported adverse events were documented. Intra-oral photos, saliva samples, plaque index, gingival index, gingival crevicular fluid samples, and plaque samples were obtained. Prior to brushing, the subjects were instructed to place 2ml / 2.6 g of their allocated toothpaste into the stent, and then place the stent in the assigned area. The stent remained in place until the two minutes of brushing was completed. Repeat samples of gingival crevicular fluid were obtained at 1, 2, 4, and 6 hours after the initial brushing. The subjects were informed to sustain their study home care regimen until the next appointment. At this time, the subjects received an incentive payment of $100.

3.17 Experimental Gingivitis Phase – Day 21 Study Appointment

Prior to this appointment, the subjects were informed to abstain from brushing their teeth for 10-12 hours. The medical history and accompanying medications were reviewed and updated if necessary, and report of adverse events was documented. Intra-oral photos, saliva samples, plaque index, gingival index, gingival crevicular fluid samples, and plaque samples were obtained. Prior to brushing, the subjects were instructed to place 2ml / 2.6 g of their allocated toothpaste into the stent, and then place the stent in the assigned area. The stent remained in place until the two minutes of brushing was completed. Repeat samples of gingival crevicular fluid were obtained at 1, 2, 4, and 6 hours after the initial brushing.

The subjects were given a prophylaxis of their entire dentition, and their stents were collected. To determine the level of study compliance, unused toothpaste was collected. If more than 60 percent of the assigned toothpaste remained, the subject was considered non-compliant, and faced dismissal from the study.[84] Participants who exhibited compliance with their allocated toothpaste received an additional $100 incentive payment.

3.18 Recovery Phase – Day 35 Follow up Contract

Prior to this final appointment, the subjects were informed to abstain from brushing their teeth for 10-12 hours. The medical history and accompanying medications were reviewed and updated if necessary, and reported adverse events were documented. Intra-oral photos, saliva samples, plaque index, gingival index, gingival crevicular fluid samples, and plaque samples were obtained. A sole gingival crevicular fluid sample was obtained from the mesiobuccal or distobuccal surface of two mandibular teeth, and the plaque samples were acquired from the same areas that the GCF samples were obtained. Participants were given a $300 incentive payment.

3.19 Study Limitations

Although the design and methodology of this study were carefully considered by the investigators, there were potential limitations and obstacles of this study. The inclusion and exclusion criteria narrowed the pool of eligible candidates due to various reasons. The time commitment required at MCOHR on Study Appointment Days 14 and 21 may have eliminated potential subjects who could not commit due to school, work, family, or other obligations. A sample size calculation was not completed for this study; the

estimate of thirty subjects was determined from results founded from a previous experimental gingivitis study. Therefore, if a difference existed, it may not have been detected because it could not be assured that there was a large enough sample for adequate statistical power.[85] Although the sample size of thirty subjects was reached, there was no guarantee that the subjects would remain for the entire duration of the study, or comply with the home care protocol. Additionally, subjects could have missed a scheduled appointment, began a new medication, developed an adverse reaction, or underwent emergency medical or dental treatment. All of these circumstances could have interfered with the study parameters and outcomes. To increase subject compliance, substantial effort was made to ensure the subjects were informed of the study requirements, and an incentive payment was given.

3.20 Statistical Analysis

Statistical analysis was conducted using SPSS. Descriptive statistics were constructed from the demographic data, and included the average age of the subjects, gender, the male to female ratio, and ethnicity. The descriptive statistics were then broken down, and basic statistical analyses using both independent samples t-tests and paired t-tests were conducted to determine if there was a difference between the two groups in this data. Additional tests were conducted for analysis of the effect of Colgate Total® toothpaste on microbial pathogens in the plaque biofilm samples as compared to the Colgate® Cavity Protection. Both independent t-tests and paired samples t-tests were used to assess each phase of the research study, including Baseline, Day 14, 21, and 35. Finally, a full linear mixed model analysis was performed to combine all of the time points and control variables in one model.

The forty microbial pathogens were analyzed using the Wilcoxon Signed Rank test. This test was used to examine differences in each pathogen from Day 0 to Day 14, Day 0 to Day 21, Day 14 to Day 21, and Day 21 to Day 35 in both the Colgate Total® and Colgate Great Regular Flavor groups. The Wilcoxon Rank Sum test was used to evaluate if the changes at each of these four paired time points revealed significant differences between the two groups.

3.21 Human Subjects

Thirty human subjects were recruited for this study. All subjects were randomly assigned into an arm of the study (treatment or control) and side of mandibular stent (right or left).

3.22 IRB Approval

This study was approved by the University of Michigan Medical School Internal Review Board (IRBMED). The current approval period is from 10/22/2013 - 10/21/2014. There was more than minimal risk to the subjects, and no direct benefit was obtained from study participation. Certain research team members were required to complete the PEERRS mandatory training on the protection of human subjects before participating in the study.

3.23 Inclusion Criteria

- Race –all races were included in the study
- Gender – male or female
- Age – between 18 and 40 years

• Dentition – a minimum of twenty permanent teeth was required for participation

• Probing Pocket Depth must be ≤ 4 mm in all sites

• Mean Clinical Attachment Level must be ≤ 2 mm on each tooth

• Bleeding on Probing must be ≥ 30% at Day 14 Study Visit

• Consent form must be read, comprehended, and signed

• Subjects must be willing to comply with all study procedures

Eligible study subjects must present with BOP of ≤ 10% at Day 0 Study Visit. Those who did not meet eligibility requirements were requested to return in 2 weeks for a follow up assessment appointment. If they did not meet the requirement of BOP of ≤ 10% at the subsequent appointment, they were excluded from participation in the study.

3.24 Exclusion Criteria

• Medical history exclusions - subjects with immune system diseases, conditions that may have affected the study outcome such as systemic infections, unstable psychiatric or neurological disorders, and a history of drug abuse or alcoholism

• Subjects taking certain medications such as those that are known to affect periodontal status including immunosuppressives, Depo-Provera contraceptive injection, phenytoin, anti-inflammatory medications, and calcium antagonists anti-convulsives will be excluded, as well as subjects who have begun oral contraceptive use in the past three months, or plan to begin using them during the study time period

• Known hypersensitivity to or an oral allergy to any of the ingredients in the dentifrices used in the study

• Subjects with a history of recent antibiotic therapy (within 3 months of the baseline appointment), and those requiring pre-medication for total joint replacement or infective endocarditis prophylaxis

• Subjects who had used antiseptics to control the formation of dental plaque within 30 days of the baseline visit

• Current smokers, previous smokers who quit smoking less than one year ago, or those with a pack year history of greater than or equal to 10 (the pack year calculation will be assessed by multiplying the average number of cigarette packs smoked per day by the number of years smoked)

• Subjects with a positive urinalysis results of Cotinine

• Subjects undergoing current periodontal or orthodontic treatment

• Subjects with unrestored carious lesions or defective restorations that could deteriorate due to the absence of mechanical plaque removal

• Women who were pregnant or lactating

3.25 Sources of Research Material

The sources of research material acquired from the study subjects included clinical periodontal measurements (PPD, CAL, and FGM), saliva samples, gingival index, plaque index, gingival crevicular fluid samples, plaque samples, biomarker analysis, and

microbial analysis. The measurements, samples, and analysis results were specifically used for research purposes pertaining to this study.

3.26 Recruitment of Study Subjects

Recruitment of subjects began after study approval was obtained. Recruitment was done through public advertising on the MCOHR website and at the MCOHR office location, the UM Human Research Recruiting Registry (UMClinicalStudies.org), and posted IRB approved flyers at various locations on the University of Michigan Campus, including the Dental School (Appendix A). Indexed cards were attached to the flyers with contact information for interested participants. Previous MCOHR clinical research study participants were contacted and informed of the opportunity to participate in this study (Appendix F). All interested participants were initially screened by phone (Appendix C). Study recruitment approach was impartial, and represented the sample population needed for the study because of the diverse University community in which the advertisement occurred. All ethnicities, and both males and females, were coequally eligible for participation, and included a percentage of individuals from the University of Michigan School of Dentistry.

3.27 Consent Procedures

A comprehensive written informed consent was obtained from all study subjects. A research study team member such as the Principal Investigator, Co-Investigator, Study Coordinator, or Research Assistant explained the study objective, all information pertaining to the study, and informed consent to the participants (Appendix B).

All subjects were informed that their participation was voluntary, and they were free to exit the study at any point. Subjects were informed that if they decide to leave the study prior to completion, there would be no penalty inflicted upon them, and no entitled benefits would be lost. Subjects that discontinued participation received a free prophylaxis and follow up appointment for evaluation of their gingival tissues.

Subjects were informed of the required number of appointments, the inclusion and exclusion criteria, what procedures would be conducted at each day of the study, the amount of time the study would require, when their participation would end, information about the benefits and risks of their participation, what to do if adverse events occurred, their options if they decide not to participate, what to do if they decided to discontinue participation in the study, reasons why the researchers may remove them from the study, financial information related to the study including incentive payment, and who may profit from the study (Colgate-Palmolive Company).

Time was allotted to answer all questions the potential subjects may have had. All subjects were required to sign the *Consent to be Part of a Research Study* document. All subjects were given a copy of the signed informed consent document. The informed consent document was approved by the University of Michigan IRBMED, and the documentation of consent was required for participation.

3.28 Potential Risks

The primary risk of conducting research with specimen samples or secondary data is breach of privacy or confidentiality. This presents the potential to result in financial, legal, social/reputation, or psychological harm to the study participant.

The experimental gingivitis model has been used safely with generally minimal risks to participants. Discontinuation of oral hygiene can result in bleeding gingiva, accumulation of plaque biofilm, and halitosis. The results of previous studies demonstrate that these effects can be completely reversed once proper oral hygiene has resumed. Gingival health can be restored within seven days of completing a 21 day period of absence of oral hygiene during a research study.

There are minimal risks involved with collection of saliva, plaque, and gingival crevicular fluid samples, and assessment of clinical periodontal parameters. There may be minor discomfort with probing and plaque collection during the gingivitis phase of the study, and from scaling and polishing during the prophylaxis. Additionally, gingival bleeding may occur during sample collection or the prophylaxis. Because of the adverse effect of discontinuing oral hygiene on decayed teeth or periodontal disease, subjects with existing dental needs will be excluded from the study.

Study participants could have developed side effects or adverse reactions to the allocated study toothpaste, including irritation in the oral cavity. Participants were questioned at each appointment to determine the occurrence of any such events. Additionally, the subjects were informed that all research studies have the potential for unknown or unexpected risks, and that every effort would be made to prevent or minimize these risks.

3.29 Protection Against Risks

This research study involved the access, collection, use, and disclosure of the University of Michigan protected health information (PHI), which is a UM HIPAA covered

component. The study had a Data and Safety Monitoring Plan. Risks to participants were minimized by meticulous screening and observation of the subjects by the investigators and research staff throughout the duration of the study. The study investigators were responsible for the documentation of all adverse events or reactions during the study. This documentation was then be forwarded to the Product Safety Assurance Department. Colgate-Palmolive Company was responsible for the costs related to medical treatment if the subject sought treatment as required by the investigator or sponsoring company. If an adverse event occurred, the subjects were informed to contact the Principal Investigator, or MCOHR staff, and in case of a medical emergency, their physician or local poison control center. Adverse events that were serious or had the potential to become serious required additional documentation and follow-up. The investigators were responsible for remaining abreast of all adverse events until they were resolved.

To protect the subject's privacy and confidentiality, all eligible participants received an assigned study number, which will be used for the entire study. For additional protection, all CRF form and sample labels exhibited the study number and subject's initials. The data and records acquired from this study were protected against inappropriate disclosure or use by being kept in a locked office with a locked storage unit or cabinet. This area had restricted access to limited number of individuals. Computer information was kept on a secure laptop that required individual ID and password protection. Routine electronic backup was performed to ensure computerized data was not lost, and network restrictions were in place. At the conclusion of the study, the data will be retained for the purpose of record keeping for two years after the

completion of the study. All subjects study binders will be stored, including those that discontinue participation in the study.

3.30 Potential Benefits

The risks to the study participants were reasonable in relation to the anticipated benefits of this study. The procedures defined in this study were documented in several other studies with the same design. The results of this study provided evidence in regard to the effect of Colgate Total® toothpaste on the gingival inflammatory response, and this information could invariably be used to improve the public's oral health. Plaque induced gingivitis has the potential to progress into periodontitis, which is a chronic, oral disease that affects millions of adults.[26] The emergent relationship between periodontal disease and systemic health supports the potential benefits and outweighs the risks of this research.

CHAPTER IV

RESULTS

4.1 Enrollment Retention and Adverse Events

A CONSORT diagram of study participants is presented (Figure 4.1). Of the 580 subjects who were interested in the study, 175 completed the phone screen. Seventy-eight of the subjects who completed the phone screen presented to the Michigan Center for Oral Health Research for the initial screening appointment. After informed consent was obtained, the subjects completed the screening appointment. At the screening appointment, thirty-eight subjects failed to meet the inclusion / exclusion criteria, and forty moved to baseline. Of the forty subjects who moved to baseline, one failed to show for the baseline appointment, one failed to qualify, two failed to show at the re-baseline, and six failed to qualify for the study at re-baseline. One subject developed a carious lesion during the experimental gingivitis phase (Day 21), and no longer qualified for participation. The subject was advised to schedule an appointment with her dentist for restorative care, and was dismissed from the study. An additional subject withdrew after the baseline appointment due to personal reasons. Two more subjects were then recruited, and a total of thirty subjects were randomized into the experimental or control

groups following the randomization chart (see Appendix E), and proceeded to complete the study.

In addition to the carious lesion, other insignificant adverse events were reported (see Appendix G), including tissue sloughing (two subjects in the Colgate Great Regular Flavor arm), herpetic lesions (two subjects in the Colgate Total® arm), apthous ulcers (one in the Colgate Great Regular Flavor arm and three subjects in the Colgate Total® arm), TMJ soreness (one subject in the Colgate Total® arm), tingling sensation in the tongue (one subject in the Colgate Total® arm, the same subject also developed an apthous ulcer), and toothbrush trauma (one subject in the Colgate Total® arm). All of the reported adverse events were resolved by the end of the study. The outcome of the dismissed subject with the carious lesion remains unknown.

4.2 Compliance

To determine the level of subject compliance, the initial weight of each toothpaste tube was documented in the subject's chart at the Baseline (Day 0) appointment. At the Day 21 Study Visit, unused toothpaste was collected and re-weighed. If more than 60 percent of the assigned toothpaste remained, the subject would be considered non-compliant, and face dismissal from the study. Based on the weight of the unused toothpaste, all thirty subjects demonstrated compliance with the study protocol.

4.3 Background Characteristics

Descriptive statistics for the thirty study participants are provided in Table 1, with the demographic information of the subjects at baseline classified according to dentifrice group. Of the thirty subjects who qualified for the study, 16 subjects were randomized

into the Colgate Total® group (seven males (43.8%) and nine females (56.3%), mean age: 26.1 ± 5.2 years old), and fourteen subjects were randomized into the Colgate Great Regular Flavor group (two males (14.3%) and twelve females (85.7%), mean age: 27.1 ± 5.2 years old). Independent samples t-tests were performed to investigate if a significant difference in age existed between the two groups, and no significant differences were observed between groups (p= .600) (Table 1). Furthermore, the results of Fisher's Exact Test revealed no significant differences between groups with respect to gender (p=.118) (Table 1).

Caucasians comprised the largest ethnicity in each group, with N=11 (68.8%) in the Colgate Total® group, and N = 9 (64.3%) in the Colgate Great Regular Flavor group. Asians constituted the next largest ethnic group, with N =2 (12.5%) in the Colgate Total® group, and N= 2 (14.3%) in the Colgate Great Regular Flavor group. The remaining ethnicities, African American, Hispanic, and Other Race had N =1 subject of each ethnicity in both groups, with the Colgate Total® sample 6.3% of each ethnicity, and the Colgate Great Regular Flavor sample 7.1% of each ethnicity. The results of Fisher's Exact Test revealed no significant differences between the groups with respect to ethnicity (p= 1.00) (Table 1).

4.4 Clinical Measures

At the screening appointment (Day -14), the subjects in the Colgate Total® group demonstrated a mean CAL ± SD of 0.76 mm ± 0.25 mm, and a mean BOP ± SD score of 0.47 percent ± 0.11 percent. Comparatively, the subjects in the Colgate Great Regular Flavor group had a mean CAL ± SD 0.75 mm ± 0.34 mm, and a mean BOP ±

SD score of .49 percent ± 0.13 percent at the screening appointment. Independent samples t-tests were performed to investigate if significant differences in CAL and BOP existed between the two groups, and no significant differences were noted between groups (p=.931 for CAL and p=.656 for BOP) (Table 1).

The study inclusion criteria required the subjects in both groups to demonstrate a BOP score of greater than or equal to 30 percent at Day -14, and a BOP score of less than or equal to10 percent at Day 0. A paired samples t-test was performed to determine if overall significant differences existed between the BOP scores on Day -14 and Day 0 pooling both the Colgate Total® and Colgate Great Regular Flavor groups together, and a significant difference was noted (p<.001) (Table 2). A series of paired samples t-tests were conducted to determine if significant differences existed in the BOP scores at Day -14 and Day 0 within both groups (Table 2). A significant decrease was noted in both the Colgate Total® group (p <.001) and the Colgate Great Regular Flavor group (p < .001) (Table 2).

Descriptive statistics of the Gingival and Plaque Indices are provided in Table 3, with the data classified according to dentifrice group and study appointment. At baseline (Day 0), the subjects in the Colgate Total® group demonstrated a mean GI score of 0.32 ± SD 0.13, and a mean PI score of 0.44 ± SD 0.29 (Table 3). Comparatively, subjects in the Colgate Great Regular Flavor Group demonstrated a mean GI score of 0.24 ± SD 0.16, and a mean PI score of 0.38 ± SD 0.26 at baseline (Table 3). On Day 14, the Colgate Total® groups GI also increased to a mean score of 1.40 ± SD 0.29, and the PI increased to a mean score of 1.51 ± SD 0.31 (Table 3). Similarly, the Colgate Great Regular flavor groups scores increased, with the mean GI score 1.30 ± SD 0.25, and

the mean PI score 1.19 ± SD 0.26 (Table 3). The GI and PI scores in both groups culminated on Day 21, with the mean GI score of Colgate Total® group 1.54 ± SD 0.30, and a mean PI score of 1.85 ± SD 0.43 (Table 3). The Colgate Great Regular Flavor group presented a mean GI score of 1.53 ± SD 0.30, and a mean PI score1.76 ± SD 0.41 on Day 21 (Table 3). By the Day 35 follow up appointment, both groups demonstrated a marked decrease in both GI and PI, with slightly higher scores than the Day 0 appointment. The mean GI of the Colgate Total® group was 0.48 ± SD 0.27, and the mean PI score was 0.56 ± SD 0.19 (Table 3). The Colgate Great Regular Flavor group had corresponding GI scores of 0.40 ± SD 0.28, and a mean PI score of 0.44 ± SD 0.24 at the Day 35 study appointment (Table 3). Longitudinal plots of the GI and PI measurements are depicted in Figure 4.2 and Figure 4.3, respectively.

A series of independent samples t-tests were conducted to ascertain if significant differences existed between the two groups in relation to the Gingival Index and Plaque Index scores at each time point. No significant differences were noted between groups in regard to the GI and PI ($p > 0.05$) at each time point with the exception of the Day 14 Plaque Index ($p = .005$), with the Colgate Total® group demonstrating more plaque accumulation at this study time point (Table 3).

Paired samples t-tests were conducted to determine if significant overall differences existed in the Plaque and Gingival Index scores for both groups (merged data) over time (Table 4). The Plaque and Gingival Index scores were compared by paired study visit (Day 0 to Day 14, Day 0 to Day 21, Day 0 to Day 35, Day 14 to Day 21, and Day 21 to Day 35) (Table 4). The results of the paired samples test revealed that the GI and PI scores increased significantly from Day 0 to Day 21, and significant differences were

observed at all paired study visits for both the GI and PI ($p < .05$), except for the PI score for Day 0 to Day 35 ($p=.091$) (Table 4).

A series of paired samples t-tests were conducted to determine if significant differences existed in the Plaque and Gingival Index scores within both the Colgate Total® and Colgate Great Regular Flavor groups (Table 5). The scores assessed on Day 0 and Day 14, Day 0 and 21, Day 0 and Day 35, Day 14 and Day 21, and Day 21 and Day 35 were paired for comparison. There was a noted increase in the mean difference of both the GI and PI scores from Day 0 to Day 14 and Day 0 to Day 21 in both the Colgate Total® and Colgate Great Regular Flavor groups (Table 5). The results of the paired samples t-tests demonstrated a significant difference between the GI and PI scores in both groups from Day 0 to Day 14, Day 0 to Day 21, Day 14 to Day 21, and Day 21 to Day 35 ($p < .05$) (Table 5). There was not a significant difference between the PI scores from Day 0 to Day 35 in either group; however, the GI score for the Colgate Total® group and the Colgate Great Regular flavor group increased significantly ($p <.001$ and $p = .004$, respectively) (Table 5).

4.4A Longitudinal Intergroup Comparisons of Gingival Index and Plaque Index Over Time

A linear mixed model was used separately for both PI and GI outcomes to combine both time and group information into a statistical model that accounts for the dependent nature of the data. During the experimental gingivitis phase, a statistically significant increase in both plaque biofilm accumulation (Plaque Index) and gingival inflammation (Gingival Index) was noted at each time point compared to Day 0 when controlling for

group (Day 14, Day 21, and Day 35) ($p < 0.05$), with the exception of the PI score on Day 35 ($p = .216$) when controlling for group (Table 6). A statistically significant difference between the Colgate Total® and the Colgate Great Regular Flavor groups was demonstrated for the PI outcome when controlling for time, with on average the Colgate Great Regular Flavor group demonstrating 146 units less PI than the Colgate Total Group ($p = .031$) (Table 6). However, there was not a statistical difference between groups for the GI outcome when controlling for time ($p = .283$) (Table 6).

4.5 Microbial Pathogen Analysis of Four Pathogens

Analysis of four of the forty bacterial microbes analyzed using the checkerboard DNA-DNA hybridization technique are depicted in Table 7, with the data classified according to dentifrice group, pathogen, and study appointment. The pathogens chosen for analysis include *S. mitis, A. israelii, P. nigrescens, and F. nucleatum ss polymorphum. S. mitis* were chosen because of their inherent association with gingival health, and *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* are pathogens associated with gingivitis. Quantities of each pathogen taken from the two sites per visit were merged and averaged for the data analysis.

Relative to the pathogen *S. mitis,* the Colgate Total® group initially demonstrated a mean of 2.61 ± SD 2.09, and the Colgate Great Regular Flavor group revealed a mean of 2.02 ± SD1.76 at Day 0 (Table 7). Both groups increased at Day 14 (Colgate Total® group mean 3.40 ± SD 2.63; Colgate Great Regular Flavor group mean 2.62 ± SD 1.61); however, at Day 21, the Colgate Total® group demonstrated a decrease (mean 2.98 ± SD 1.95), but the Colgate Great Regular Flavor group continued to increase

(mean 2.79 ± SD 1.76) (Table 7). By the Day 35 follow up appointment, both groups began to decrease, with the Colgate Total® group mean 2.72 ± SD 2.26, and the Colgate Great Regular Flavor group mean 1.69 ± SD 2.03 (Table 7).

The pathogen *A. israelii* showed similar results to *S. mitis* on Day 0 and 14. The Colgate Total® group's mean was 2.54 ± SD 1.67, and the Colgate Great Regular Flavor group demonstrated a mean of 2.31 ± SD 3.06 at Day 0 (Table 7). On Day 14, the Colgate Total® group increased to a mean pathogen level of 4.90 ± SD 3.40, and the Colgate Great Regular Flavor group increased to a mean of 6.81 ± SD 6.57 (Table 7). In contrast to the *S. mitis* pathogen, the Colgate Total® group continued to increase (mean 5.67 ± SD 3.85), but the Colgate Great Regular Flavor group level decreased on Day 21 (mean 5.89 ± SD 4.83) (Table 7). By Day 35, both groups markedly decreased, with the Colgate Total® group's mean 2.92 ± SD 2.83, and the Colgate Great Regular Flavor group's mean 2.07 ± SD 2.44 (Table 7).

The pathogen *P. nigrescens* demonstrated an increase from Day 0 to Day 21, and a notable decrease at the Day 35 follow up appointment in both groups. At Day 0, the Colgate Total® group had a mean level of 0.94 ± SD 2.21; comparatively, the Colgate Great Regular Flavor group had a mean level of 0.54 ± SD 1.03 (Table 7). The mean level of the Colgate Total® group on Day 14 was 3.95 ± SD 4.77, and the Colgate Great Regular flavor group mean level was 5.12 ± SD 4.73 (Table 7). At the Day 21 appointment, the Colgate Total® group level of *P. nigrescens* increased more (mean 7.88 ± SD 6.13) when compared to the Colgate Great Regular flavor group (mean 6.74 ± SD 5.22) (Table 7). At the Day 35 follow up appointment, both groups demonstrated

a distinct decrease, with the Colgate Total® group mean 1.13 ± SD 1.82, and the Colgate Great Regular Flavor group mean 0.85 ± SD 1.43 (Table 7).

The final pathogen analysis was conducted on *F. nucleatum ss polymorphum,* and the results were similar to *P. nigrescens*, with both groups increasing from Day 0 to Day 21, and decreasing by the Day 35 follow up appointment. On Day 0, the Colgate Total® group demonstrated a mean level of 1.75 ± SD 1.85, and the Colgate Great Regular Flavor group mean level was 0.99 ± SD 1.33 (Table 7). The levels increased at Day 14, with the Colgate Total® group's mean 3.55 ± SD 3.45, and the Colgate Great Regular Flavor group mean was 2.96 ± SD 2.04 (Table 7). The levels of both groups further increased at Day 21, with the Colgate Total® group mean culminating at 5.98 ± SD 5.42, and the Colgate Great Regular Flavor group mean 4.25 ± SD 2.18 (Table 7). Similar to the *P. nigrescens* pathogen, both groups notably decreased at the Day 35 appointment, with the Colgate Total® group mean level of *F. nucleatum ss polymorphum* measuring at 1.04 ± SD 0.92, and the Colgate Great Regular Flavor group mean level 1.27 ± SD 1.61 (Table 7).

A series of independent samples t-test were conducted to ascertain if significant differences between the two groups exist at each time point for all four pathogens. No significant differences were observed between the groups for any of the four pathogens at any time point ($p > 0.05$) (Table 7).

Paired samples t-tests were conducted to determine if significant differences existed in the four microbial pathogens (Table 8). The pathogen averages acquired on were compared by paired study visit (Day 0 to Day 14, Day 0 to Day 21, Day 0 to Day 35,

Day 14 to Day 21, and Day 21 to Day 35 (Table 8). The results of the paired samples test revealed significant differences for all four pathogens from Day 0 to Day 14 ($p < .05$) (Table 8). In addition, significant differences were noted for *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* on Day 0 to Day 21 ($p < .001$); however, *S. mitis* was not significant ($p = .179$) (Table 8). The mean difference level of the pathogens *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* increased from Day 0 to Day 21 in both the Colgate Total® and Colgate Great Regular Flavor groups; however, the pathogen *S. mitis* demonstrated an increase from Day 0 to Day 21 solely in the Colgate Great Regular group (Table 7). Although there was an initial increase of *S. mitis* from Day 0 to Day 14 in the Colgate Total® group, the mean difference of the level of pathogen decreased from Day 14 to Day 21 (Table 7). No significant differences were noted for any of the four pathogens on the Day 0 to Day 35 comparison ($p > .05$) (Table 8). The Day 14 to Day 21 comparison revealed significant differences for *P. nigrescens* and *F. nucleatum polymorphum* ($p < .05$); however, *S. mitis* and *A. israelii* were not significant ($p = .709$ and $p = .965$, respectively) (Table 8). Significant differences were noted for all pathogens ($p < .001$) with the exception of *S. mitis* ($p = .135$) from Day 21 to Day 35 (Table 8).

A series of paired samples t-tests were conducted to determine if significant differences existed in the four microbial pathogens within both groups (Table 9). The pathogen averages acquired on Day 0 and Day14, Day 0 and 21, Day 0 and Day 35, Day 14 and Day 21, and Day 21 and Day 35 were paired for comparison. The results of the paired samples t-tests demonstrated a significant increase between *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* in both groups from Day 0 to Day 14 (p

< .05) (Table 9); however, *S.mitis* was significant only in the Colgate Total® group (p= .008) (Table 9). The Day 0 to Day 21 pairing also revealed a significant increase between *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* in both groups (p <.05) (Table 9). There was not a significant difference with *S. mitis* in either group (p=.512, p= .229) (Table 9). In addition, there was not a significant difference in any of the four pathogens in the Day 0 to Day 35 pairing for either group (Table 9). However, the Day 14 to Day 21 pairing revealed a significant increase with *P. nigrescens* and *F. nucleatum ss polymorphum* in the Colgate Total® group (p <.05), and the pathogen *F. nucleatum ss polymorphum* was near significance in the Colgate Great Regular Flavor group (p=.055); the remaining pathogens were not significant in either group (p>.05) (Table 9). Finally, the Day 21 to 35 pairing revealed significant decreases for all of the pathogens in both groups (p <.05) with the exception of *S. mitis* (p=.547 in the Colgate Total® group, and p =.177 in the Colgate Great Regular Flavor group) (Table 9).

4.6 Microbial Analysis of all Forty Pathogens

The forty microbial pathogens evaluated by the previously described DNA-DNA Hybridization technique were statistically analyzed, and categorized into the seven complexes as previously described by Socransky et al (Figure 4.2).[62] Wilcoxon Rank Sum and Wilcoxon Signed Rank tests were conducted to ascertain statistical significance in each group and between groups. The bacterial counts of each complex for each subject at Baseline were classified by dentifrice group, and are depicted in Table 10. Total bacterial counts increased significantly from baseline to Day 21 and decreased significantly from Day 21 to Day 35 (p<0.05) (Figure 4.2). All microbial complexes demonstrated statistically significant increases from Day 0 to Day 21 in both

the Colgate Total® and the Colgate Great Regular Flavor Groups (Figure 4.2). The results of the analysis of all forty pathogens revealed limited significant differences between groups at certain time points, and the proliferation of certain pathogens was noted. These results prompted further evaluation of all forty pathogens to test the hypothesis in this thesis, rather than the sole four pathogens initially chosen.

4.6a Microbial Analysis of the Blue (Actinos) Complex

There were four pathogens analyzed in the Blue (Actinos) Complex, including *A.gerencseriae, A.israelli, A.naeslundi,* and *A.oris.* From Day 0 to Day 14, all four pathogens demonstrated significant increases in counts in both groups ($p < 0.05$), but no significant differences were noted between groups ($p > 0.05$) (Table 11). *A.naeslundi* was near significance ($p=0.093$), with less microbial growth in the Colgate Total® group (Table 11). In addition, Colgate Total® also demonstrated a tendency to inhibit *A. oris* proliferation ($p=0.142$) (Table 11).

From Day 14 to Day 21, no significant differences were noted in either group ($p > 0.05$) (Table 18). The Colgate Great Regular Flavor group counts decreased for all four pathogens, and the *A.israelli, A.naeslundi, A.oris* counts were near significance ($p=0.052$, $p=0.070$, $p=0.077$, respectively). Comparatively, the Colgate Total® groups counts continued to increase. In addition, when compared to the Colgate Total® group, the Colgate Great Regular Flavor group demonstrated a tendency to inhibit *A.gerencseriae* growth ($p=0.101$) (Table 18).

From Day 0 to Day 21, all four pathogens demonstrated significant increases in counts in both groups ($p < 0.05$), but no significant differences were noted between

groups (p >0.05) (Table 25). When compared to the samples taken on Day 21, the Day 35 counts revealed significant decreases in both groups (p <0.05), and no significant differences were noted between groups (p>0.05) (Table 32).

4.6b Microbial Analysis of the Yellow Complex

There were five pathogens analyzed in the Yellow Complex, including *S.gordonii, S.intermedia, S.mitis, S.oralis*, and *S.sanguinis.* From Day 0 to Day 14, *S.gordonii, S.intermedia,* and *S.sanguinis* demonstrated significant increases in counts in both groups, and *S. mitis* increased significantly solely in the Colgate Total® group (p < 0.05) (Table 12). *S. oralis* did not significantly change in either group (Table 12). No significant differences were noted between groups (p >0.05) (Table 12).

From Day 14 to Day 21, the Colgate Great Regular Flavor group revealed a significant decrease for the *S.sanguinis* pathogen (p=0.035); *S.gordonii* was nearly significant (p=0.068) (Table 19). No significant differences were noted in the Colgate Total® group, and no significant differences were noted between groups for any of the five pathogens (p> 0.05) (Table 19).

From Day 0 to Day 21, the *S.gordonii* increase in the Colgate Total® group was near significance (p=0.051), and the *S.intermedia* counts increased significantly in both groups (p=0.035 in the control group and p=0.003 in the test group) (Table 26). There was no significant difference between groups in any of the five pathogens (p>0.05) (Table 26).

On Day 35, the pathogen counts in each group decreased from the Day 21 amounts (Table 33). Significant decreases were noted in *S.intermedia* in the Colgate Great

Regular Flavor group (p=0.004), and the Colgate Total® group was near significance (p=0.058) (Table 31). Both groups exhibited a tendency to prevent *S.oralis* proliferation (p=0.153 in the control group and p=0.159 in the test group) (Table 33). In addition, when compared to the Colgate Total® group, the Colgate Great Regular Flavor group demonstrated an inclination to inhibit *S.mitis* growth (p=0.070) (Table 33).

4.6c Microbial Analysis of the Purple Complex

There were two pathogens analyzed in the Purple Complex, *A.odontolyticus* and *V.parvula.* From Day 0 to Day 14, significant increases were noted for both pathogens in both groups ($p<0.05$); however, there was no significant difference between groups ($p>0.05$) (Table 13). The quantities of both pathogens continued to increase in the Colgate Total® group, and decreased in the Colgate Great Regular Flavor group from Day 14 to Day 21 (Table 20). With the exception of *V. parvula* in the Colgate Total® group which was near significance (p=0.083), these changes were not statistically significant ($p>0.05$) (Table 20). There were no significant differences between groups ($p>0.05$) (Table 20).

Both pathogens in the Purple Complex increased from Day 0 to Day 21, with significant increases in *A.odontolyticus* in both groups ($p<0.05$); however, the increase of the quantity of *V.parvula* was only significant in the Colgate Total® group (p=0.002) (Table 27). There were no significant differences between groups ($p>0.05$) (Table 27). At Day 35, both pathogens demonstrated a decrease in quantity compared to Day 21, with significant decreases for *A.odontolyticus* in both groups ($p<0.05$); however the decrease of the quantity of *V.parvula* was only significant in the Colgate Total® group,

which was similar to the Day 0 to Day 21 differences (Table 34). There were no significant differences between groups ($p>0.05$) (Table 34).

4.6d Microbial Analysis of the Green Complex

There were five pathogens analyzed in the Green Complex, including *A.actinomycetemcomitans*, *C.ochracea, C.gingivalis, C.sputige*, and *E.corrodens.* All of the pathogens demonstrated an increase in quantity from Day 0 to Day 14, with the exception of *A.actinomycetemcomitans* in the Colgate Total® group (Table 14). Significant increases were noted for *C.ochracea* and *C.gingivalis* in both groups ($p<0.05$), and *C.sputige* significantly increased in the Colgate Total® group ($p=0.039$), and was near significance in the Colgate Great Regular Flavor group ($p=0.078$) (Table 14). The sole significant difference between groups was with *A.actinomycetemcomitans,* with the Colgate Total® dentifrice demonstrating a tendency to inhibit *A.actinomycetemcomitans* proliferation ($p=0.012$) (Table 14).

Analysis of the changes from Day 14 to Day 21 revealed a continual increase in *A.actinomycetemcomitans* and *C.ochracea* quantities in both groups; an increase in *C.gingivalis* and *E.corrodens* in the Colgate Great Regular Flavor group, and *C.sputige* in the Colgate Total® group; however, *C.gingivalis* and *E.corrodens* decreased in the Colgate Total® group, and *C.sputige* decreased in the Colgate Great Regular Flavor group, with no significant differences in either group ($p>0.05$) (Table 21). The increase of *A.actinomycetemcomitans* was near significance in the Colgate Total® group ($p=0.083$)(Table 21). There were no significant differences in the microbial quantity increases between the two groups ($p>0.05$) (Table 21). With the exception of

A.actinomycetemcomitans in the Colgate Total® group, the quantities of pathogen also increased from Day 0 to Day 21, with significant increases noted for *C.ochracea* and *C.gingivalis* in both groups ($p<0.05$), and *C.sputige* in the Colgate Total® group ($p=0.002$) (Table 28). The increase of *A.actinomycetemcomitans* in the Colgate Great Regular Flavor group was near significance ($p=0.068$) (Table 28). Similar to the Day 0 to Day 14 changes, the sole significant difference between groups was with *A.actinomycetemcomitans,* with the Colgate Total® dentifrice demonstrating a tendency to inhibit *A.actinomycetemcomitans* proliferation ($p=0.038$) (Table 28).

At Day 35, all five pathogens demonstrated a decrease in total quantity from Day 21, with significant decreases noted in *C.ochracea* and *C.sputige* in both groups ($p<0.05$) (Table 35). *C.gingivalis* demonstrated a significant decrease in the Colgate Great Regular Flavor group ($p=0.035$), and was near significant in the Colgate Total® group ($p=0.093$) (Table 35). *A.actinomycetemcomitans* and *E.corrodens* were near significance in the Colgate Great Regular Flavor group ($p= 0.058$ and $p=0.078$, respectively), but did not significantly decrease in the Colgate Total® group ($p= 0.144$ and $p=0.231$, respectively) (Table 35). There were no significant differences between groups ($p>0.05$) (Table 35).

4.6e Microbial Analysis of the Orange Complex

There were twelve pathogens analyzed in the Orange Complex, including *C.gracilis*, *C.rectus*, *C.showae*, *E.nodatum*, *F.nucleatum.ss.nucleatum*, *F.nucleatum.ss.polymorphum*, *F.nucleatum.ss.vincentii*, *F.periodonticum*, *P.micra*, *P.intermedia*, *P.nigrescens*, and *S.constellatus.* All pathogens increased in total count

from Day 0 to Day 14, and there was a significant increase in both groups for all of the pathogen counts ($p<0.05$) (Table 15). There were no significant differences between groups for any of the twelve pathogens ($p>0.05$) (Table 15). However, when comparing groups, the Colgate Total® dentifrice did demonstrate a tendency to inhibit proliferation of the *F. nucleatum.ss.nucleatum*, *F.nucleatum.ss.vincentii*, and *P.micra* pathogens ($p= 0.131$, $p=0.154$, and $p=0.077$, respectively) (Table 15).

The twelve pathogens continued to increase from Day 14 to Day 21, with the exception of *C.rectus* and *C. showae*, which decreased solely in the Colgate Great Regular Flavor group (Table 22). Significant increases were noted in both groups for the *F.nucleatum.ss.nucleatum* and *F.nucleatum.ss.vincentii* pathogens ($p<0.05$) (Table 22). Significant increases were also noted for *C.gracilis*, *E.nodatum*, *F.nucleatum.ss.polymorphum*, *F.periodonticum*, *P.micra*, *P.intermedia*, and *P.nigrescens* in the Colgate Total® group ($p<0.05$) (Table 22). *P. nigrescens, F.periodonticum,* and *F.nucleatum.ss.polymorphum* were near significance in the Colgate Great Regular Flavor group ($p=0.078$, $p= 0.068$, and $p=0.091$, respectively) (Table 22). The sole significant difference between groups was found in *P. nigrescens*, with the Colgate Great Regular Flavor dentifrice demonstrating a tendency to inhibit *P. nigrescens* proliferation more effectively when compared to Colgate Total® ($p=0.034$) (Table 22). Similarly, *P. intermedia* was near significance ($p=0.052$), with the Colgate Great Regular Flavor dentifrice demonstrating a tendency to inhibit *P.intermedia* growth more effectively when compared to Colgate Total® (Table 22).

The results from Day 0 to Day 21 mirrored the results from Day 0 to Day 14, with all pathogens increasing in total count from Day 0 to Day 21, with a significant increase in

both groups for all of the pathogen counts ($p<0.05$) (Table 29). There were no significant differences between groups for any of the twelve pathogens ($p>0.05$); however, *P. micra* was near significant, with the Colgate Total® dentifrice demonstrating a tendency to inhibit growth ($p=0.077$) (Table 29). On Day 35, all pathogens demonstrated a decrease in total quantity from Day 21, with significant decreases noted in all twelve ($p<0.05$) (Table 36). There were no significant differences noted between groups ($p>0.05$) (Table 36.)

4.6f Microbial Analysis of the Red Complex

There were three pathogens analyzed in the Red Complex, including *T.forsythia, P.gingivalis,* and *T.denticola.* All pathogens increased in quantity from Day 0 to Day 14, with significant increases were noted for all three pathogens in both groups, with the exception of *P. gingivalis* in the Colgate Total® group ($p=0.193$) (Table 16). A significant difference was noted between groups in regard to the pathogen *P.gingivalis,* with the Colgate Total® dentifrice demonstrating a tendency to inhibit proliferation of this pathogen more effectively when compared to Colgate Great Regular Flavor ($p=0.006$) (Table 16). In addition, *T. Forsythia* growth was also depressed by the Colgate Total® dentifrice when compared to the control, although this difference was not significant ($p=0.131$) (Table 16).

The three pathogens continued to increase from Day 14 to Day 21, with significant increases solely in the Colgate Total® group for all of the pathogens ($p<0.05$) (Table 23). There was no significant difference noted between the two groups for any of the pathogens ($p>0.05$) (Table 23). The quantities of these three pathogens also increased

from Day 0 to Day 21, with significant increases noted for all pathogens in both groups ($p<0.05$) (Table 30). There were no significant differences between groups ($p>0.05$) (Table 30). On Day 35, all pathogens in both groups demonstrated a significant decrease ($p<0.05$), and there were no significant differences between groups ($p>0.05$) (Table 37.)

4.6g Microbial Analysis of the Grey (Other) Complex

There were ten pathogens analyzed in the Grey (Other) Complex, including *E.saburreum, G.morbillorum, L.bucallis, N.mucosa, P.acnes, P.melaninogenica, S.anginosus, S.noxia, T.socranskii,* and *S.mutans.* All pathogens increased in total count from Day 0 to Day 14. There was a significant or near significant increase in both groups for all of the pathogen counts with the exception of *N.mucosa* ($p=0.860$ in the Colgate Total® group, and $p=0.761$ in the Colgate Great Regular Flavor group) (Table 17). In addition, *L.bucallis* did not significantly increase in the Colgate Total® group ($p=0.323$), but was near significance in the Colgate Great Regular Flavor group ($p=0.058$) (Table 17). *G.morbillorum* increased significantly in the Colgate Total® group ($p=0.016$), and was near significance in the Colgate Great Regular Flavor group ($p=0.078$) (Table 17). There were no significance differences noted between groups ($p>0.05$) (Table 17).

From Day 14 to Day 21, there were significant increases in the counts of *T.socranskii* in both groups ($p<0.05$), and significant increases in *G.morbillorum* ($p=0.044$) and *S.noxia* ($p=0.003$) solely in the Colgate Total® group (Table 24). In addition, *S. mutans* and *P. melaninogenica* were near significance in the Colgate Total® group ($p=0.074$ and

p=0.051, respectively) (Table 24). The pathogens *E.saburreum, P.acnes, and S.noxia* (Colgate Great Regular Flavor), *and L.bucallis and N.mucosa* (Colgate Total®) demonstrated decreases in pathogen counts; however, these were not significant decreases (Table 24). There were no significant differences between groups, but *S.noxia* was near significance (p=0.085) (Table 24).

The comparison between Day 0 and Day 21 revealed all pathogens increased in quantity, with significant increases noted in both groups for *E.saburreum, P.acnes, P.melaninogenica, S.anginosus, S.noxia, T.socranskii,* and *S.mutans* (p<0.05)(Table 31). The increase in *G.morbillorum* was significant in the Colgate Total® group (p=0.001) and was near significance in the Colgate Great Regular Flavor Group (p=0.068) (Table 31). The increase in *L.bucallis* was also near significance in the Colgate Great Regular Flavor group (p=0.068), but not in the Colgate Total® group (p=0.193) (Table 31). The increases in *N.mucosa* were not significant in either group (p>0.05) (Table 31). There were no significant differences noted between groups (p>0.05); however, the *S.noxia* increase was nearly significant, with the Colgate Great Regular Flavor group demonstrating the ability to inhibit proliferation of this pathogen when compared to Colgate Total® (p=0.085)(Table 31).

On Day 35, the ten pathogens demonstrated a decrease in microbial quantity in both groups when compared to Day 21, with the exception of *N.mucosa,* which increased in quantity in the Colgate Total® group (Table 38). The decreases in quantities were statistically significant (p<0.05), with the exception of *G.morbillorum* and *L.bucallis* in the Colgate Great Regular Flavor group (p=0.194 and p=0.455, respectively), and

N.mucosa quantities in both groups ($p>0.05$) (Table 38). There were no significant differences between groups ($p>0.05$) (Table 38).

CHAPTER V

DISCUSSION

5.1 Study Objective and Aim

The aim of this research project was to determine the effect of Colgate Total® toothpaste on microbial pathogens in plaque biofilm samples during the controlled environment of an experimental gingivitis model in the absence of the mechanical interruption of plaque biofilm. My hypothesis was that Colgate Total® toothpaste, when compared to a standard of care toothpaste (Colgate Great Regular Flavor), would be more effective at reducing the microbial pathogen load.

The inflammatory response from experimental gingivitis is primarily due to microbial plaque accumulation. [6,7] The experimental gingivitis model is often chosen for clinical research to evaluate the changes in the inflammatory response as the process of moving from gingival health to gingivitis occurs.[7] Clinical measures (including the Gingival and Plaque indices) were evaluated, and microbial plaque samples were obtained at Baseline, Day 14, 21 and 35. Forty microbial pathogens isolated from the plaque samples were analyzed using the checkerboard DNA-DNA hybridization technique, and the pathogens *S. mitis, A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* were initially chosen for in depth analysis for this thesis. *S. mitis* were

chosen because of their inherent association with gingival health, and *A. israelii, P. nigrescens, and F. nucleatum ss polymorphum* are pathogens associated with gingivitis. The results of the analysis of all forty pathogens revealed significant differences between groups at certain time points, and trends were noted to suppress the proliferation of certain pathogens. These results prompted further evaluation of all forty pathogens to test the hypothesis in this thesis, rather than solely the four pathogens initially chosen.

5.2 Synthesis of Research Findings – Clinical Measures and Four Pathogens

The results of this study revealed that all thirty subjects developed gingivitis, and are supported by the statistically significant differences noted in the Day 0 to Day 21 Gingival and Plaque Index scores ($p < .001$, Table 4). The documented increase in the Gingival and Plaque Index scores, combined with the weight of each subject's unused toothpaste, yields evidence that all thirty subjects were compliant with the study protocol (Table 39). Two of the pathogens associated with gingivitis (*P. nigrescens and F. nucleatum ss polymorphum*) increased in quantity in both groups from Day 0 to Day 21, and mirrored baseline on Day 35 (Table 7). The third pathogen associated with gingivitis, *A. israelii,* increased in quantity from Day 0 to Day 21 in the Colgate Total® group, however, in the Colgate Great Regular Flavor group, the quantity of *A. israelii* pathogen increased from Day 0 to day 14, then decreased from Day 14 to Day 21 (Table 7). On Day 35, the quantity of *A. israelii* mirrored baseline in both groups (Table 7). In the Colgate Total® group, the pathogen associated with gingival health, *S. mitis*, increased in quantity from Day 0 to Day 14, then decreased in quantity from Day 14 to Day 21, and mirrored baseline on Day 35 (Table 7). However, *S. mitis* increased in

quantity from Day 0 to Day 21 in the Colgate Great Regular Flavor group, and then decreased to a level less than Baseline on Day 35 (Table 7).

Statistical analysis of the clinical measures (GI and PI) did not reveal overall significant differences among groups with the exception of Day 14, where the Colgate Total® group demonstrated significantly more plaque accumulation compared to the Colgate Great Regular Flavor group (Table 3) (Figure 4.3). However, the Linear Mixed Model did reveal a significant difference for the PI outcome when controlling for time, with on average the Colgate Great Regular Flavor group demonstrating 146 units less PI than the Colgate Total Group (p=.031) (Table 6). In their landmark experimental gingivitis studies in the 1960's, Loe and colleagues used the identical Gingival and Plaque Indices uses in this study to evaluate the change in the gingival tissues and plaque accumulation in the absence of mechanical plaque removal.[50,51] Their results demonstrated an increase in both the Gingival and Plaque Index scores from baseline to the point where the absence of oral hygiene culminated.[50,51] In addition, the scores mirrored baseline when oral hygiene resumed, similar to what was revealed in this study.[50] In 1978, Syed and Loesche used a modified version of the Gingival and Plaque indices used in this study in their 21 day experimental gingivitis model evaluating the effect of plaque age.[55] The results of their study revealed an increase in plaque and gingivitis scores during the 21 day period with the absence of oral hygiene, although the increase was not as prominent from Day 14 to Day 21, similar to the results in this current study.[55] Syed and Loesche also evaluated the microbial quantities of *A. israelii, F. nucleatum, and S. mitis* over the 21 day study period, and similar to the results in this study, the quantity of *S. mitis* decreased from Day 14 to Day 21, while the

pathogens *A. israelii* and *F. nucleatum* quantities increased.[55] In 2004, in a repeat trial of previous experimental gingivitis participants who were classified as either high or low responders, Trombelli and colleagues found similar results, with both the Gingival and Plaque Index scores increasing during the 21 day study time period, and no significant differences between the two groups.[53]

5.2a Synthesis of Research Findings - All Forty Microbial Pathogens

In depth analysis of all forty microbial pathogens revealed significant differences in a limited number of pathogens, and the inhibition of certain pathogen proliferation was observed even though the statistical tests did not detect differences. The initial microbial species to establish growth on acquired pellicle include those from the Blue Complex (*Actinomyces*) Yellow Complex (*Streptococcus*), and Purple Complex (*Veillonella*). [18] Small counts of these pathogens have been isolated in healthy gingiva, and they co-aggregate with one another for survival and growth.[18] In experimental gingivitis, *Actinomyces* are the predominant microbial species, comprising up to fifty percent of the total microbial load.[27]

Small amounts of known periodontal pathogens have been detected in healthy sulci.[18,49,62] In this study, there were diminutive mean counts of known periodontal pathogens *T. forsythia. P. gingivalis, T. denticola*, and *A.actinomycetemcomitans* present at Day 0. With the exception of *A. actinomycetemcomitans*, the statistical tests were unable to detect significant differences between groups among these pathogens; however, there were trends noted of the Colgate Total® dentifrice inhibiting proliferation of certain known periodontal pathogens early in the experimental gingivitis phase.

Although the counts of the four Actinos Complex pathogens did not achieve statistical significance, the Colgate Total® dentifrice had a tendency to inhibit the proliferation of *A. naeslundi* and *A. oris* from Day 0 to Day 14, which is evident by the much lower change in bacterial count when compared to the Colgate Great Regular Flavor group (Table 11). However, the data from Day 14 to 21 of all four pathogens reveals a decrease in the Colgate Great Regular flavor group counts, while the Colgate Total® group counts continued to increase (Table 18). Although not significant, the results show that the Colgate Great Regular Flavor group had a tendency to depress the growth of the Actinos group pathogens from Day 14 to Day 21. By Day 35, all of the counts decreased significantly from Day 21; however, with the exception of *A. gerencseriae* and *A. israelii* in the Colgate Great Regular Flavor group, all of the counts were higher when compared to the Day 0 counts (Table 11 and Table 32).

The Colgate Great Regular Flavor dentifrice appeared to depress the growth of certain pathogens in the Yellow Complex more effectively. The control toothpaste inhibited proliferation of *S. mitis* from Day 0 to 14 when compared to Colgate Total, ® but there were no significant differences between groups (Table 12). Both dentifrices appeared to depress the growth of *S. oralis*, with no significant differences between groups (Table 12). From Day 14 to Day 21, the counts of *S. gordonii* and *S. sanguinis* decreased in both groups, with the Colgate Great Regular Flavor dentifrice demonstrating a tendency to inhibit growth of these two pathogens when compared to the Colgate Total® group, with no significant differences between groups (Table 19). At Day 35, all of the Yellow Complex pathogens decreased; however, when compared to the Day 0 counts, the Day 35 counts of the Colgate Great Regular Flavor group

demonstrated decreases for all pathogens with the exception of *S. gordonii*, and the Colgate Total® group counts were elevated for all pathogens with the exception of *S. oralis* (Table 12 and 33). When comparing groups, the Colgate Great Regular flavor dentifrice appeared to inhibit *S. mitis* growth from Day 21 to 35 (Table 33).

Neither dentifrice appeared to have a significant effect on the Purple Complex pathogens from Day 0 to Day 14 when comparing groups, although the Colgate Total® counts increased less (Table 13). The data from Day 14 to 21 reveals a tendency for the Colgate Great Regular Flavor dentifrice to inhibit proliferation of both pathogens, as the counts in this group decreased, and the counts in the Colgate Total® group increased (Table 20). At Day 35, the microbial counts of both pathogens decreased in both groups, and both pathogen counts were elevated when compared to the Day 0 counts (Table 13 and Table 34). The Day 0 to Day 35 comparison counts in the Colgate Total® group were higher when compared to the Colgate Great Regular Flavor group, especially in regard to *V. parvula* (Table 13 and Table 34).

Analysis of the Green Complex changes from Day 0 to Day 14 revealed that all counts increased in both groups for all five pathogens with the exception of *A. actinomycetemcomitans*, which decreased solely in the Colgate Total® group (Table 28). This decrease is compelling because *A. actinomycetemcomitans* is a known periodontal pathogen often associated with Localized Aggressive Periodontitis (formerly known as Juvenile Periodontitis).[86] Significant increases were noted for *C.ochracea* and *C.gingivalis* in both groups (Table 14). *C.sputige* significantly increased in the Colgate Total® group, and was near significance in the Colgate Great Regular Flavor group (Table 14). The sole significant difference between groups was with

A.actinomycetemcomitans, with the Colgate Total® dentifrice demonstrating a tendency to inhibit *A.actinomycetemcomitans* proliferation (Table 14).

This tendency appeared to diminish from Day 14 to Day 21, as data analysis reveals that *A.actinomycetemcomitans* and *C.ochracea* quantities increased in both groups, with the increase of *A.actinomycetemcomitans* in the Colgate Total® group near significance (Table 21). In addition, an increase in *C.gingivalis* and *E.corrodens* was observed in the Colgate Great Regular Flavor group, and *C.sputige* increased in the Colgate Total® group; however, *C.gingivalis* and *E.corrodens* decreased in the Colgate Total® group, and *C.sputige* decreased in the Colgate Great Regular Flavor group, with no significant differences in either group or between the two groups (Table 21).

At Day 35, all five pathogens demonstrated a decrease in total quantity from Day 21, with no significant differences noted between groups; however, when comparing the pathogen counts from Day 0 to Day 35, *A.actinomycetemcomitans, C.ochracea,* and *C.sputige* counts were lower and *C.gingivalis* were higher in both groups on Day 35 (Table 14 and 35). *E.corrodens* decreased in the Colgate Great Regular Flavor group and increased in the Colgate Total® group (Table 14 and Table 35). The decrease from Day 0 and Day 35 in the *A.actinomycetemcomitans* counts was greater in the Colgate Total® group (.18 vs .02) (Table 14 and 35). The triclosan-copolymer appeared to affect the proliferation of this known periodontal pathogen throughout the entire 35 Day study period.

Analysis of the Day 0 to Day 14 changes in the Orange Complex revealed all microbial counts significantly increased in both groups (Table 15). Although there were no significant differences between groups for any of the twelve pathogens, the Colgate

Total® dentifrice did demonstrate a tendency to inhibit proliferation of the *F. nucleatum.ss.nucleatum, F.nucleatum.ss.vincentii,* and *P.micra* pathogens, which is evident by the much smaller increase of these pathogen counts (Table 15).

It appears that the trends noted from Day 0 to 14 were not as prominent from Day 14 to Day 21. The twelve pathogens continued to increase, with the exception of *C.rectus* and *C. showae*, which decreased solely in the Colgate Great Regular Flavor group (Table 22). Significant increases were noted in both groups for the *F.nucleatum.ss.nucleatum* and *F.nucleatum.ss.vincentii pathogens* (Table 22). Significant increases were also noted for *C.gracilis, E.nodatum, F.nucleatum.ss.polymorphum, F.periodonticum, P.micra, P.intermedia*, and *P.nigrescens* in the Colgate Total® group, and *P. nigrescens, F.periodonticum*, and *F.nucleatum.ss.polymorphum* were near significance in the Colgate Great Regular Flavor group (Table 22). The sole significant difference between groups was found in *P. nigrescens,* with the Colgate Great Regular Flavor dentifrice demonstrating a tendency to inhibit *P. nigrescens* proliferation more effectively when compared to Colgate Total® (Table 22). Similarly, the Colgate Great Regular Flavor dentifrice demonstrated a tendency to inhibit *P.intermedia* growth more effectively when compared to Colgate Total® (Table 22).

On Day 35, all members of the Orange Complex significantly decreased from Day 21 (Table 36). Comparison of the Day 0 to Day 35 differences revealed the Colgate Total® group counts decreased from their original numbers, with the exception of *P.nigrescens*, *C. gracilis*, and *S. constellatus* (Table 15 and Table 36). However, the Colgate Great Regular Flavor group counts were higher on Day 35 than on Day 0, with the exception

of *C.gracilis*, which increased, and *S.constellatus*, which returned to its original count (Table 15 and Table 36). The differences of *F.nucleatum.ss.nucleatum, F.nucleatum.ss.polymorphum, F.nucleatum.ss.vincentii,* and *P. micra* pathogens were more notable than the rest of the pathogens (Table 15 and Table 36). A study by Socransky and colleagues revealed that the Orange Complex pathogens are late colonizers associated with the known periodontal pathogens in the Red Complex.[62] Orange Complex pathogens are also associated with increased pocket depth, and establish prior to the Red Complex pathogens.[62] These results suggest that the triclosan-copolymer formulation may have an effect on the proliferation of the Orange Complex pathogens over time, and further research may be warranted.

As previously mentioned, the three Red Complex pathogens are closely associated with periodontal disease, and are found in increased numbers in both periodontal pockets and subjects with high BOP scores.[62] All pathogens in the Red Complex increased in quantity from Day 0 to Day 14, with significant increases for all three pathogens in both groups, with the exception of *P. gingivalis* in the Colgate Total® group (Table 16). A significant difference was noted between groups in regard to the pathogen *P.gingivalis*, with the Colgate Total® dentifrice demonstrating a tendency to inhibit proliferation of this pathogen more effectively when compared to Colgate Great Regular Flavor (Table 16). In addition, *T. Forsythia* growth was also depressed by the Colgate Total® dentifrice when compared to the control, although this difference was not significant (Table 16).

Analysis of the changes in the Red Complex from Day 14 to Day 21 depict that the three pathogens continued to increase in count, with significant increases solely in the

Colgate Total® group for all of the pathogens, and there was no significant difference noted between the two groups for any of the pathogens (Table 23). Of the three pathogens, only *P. gingivalis* increased more in the Colgate Total® group when compared to the control (Table 23). Similar to the Orange Complex, it appears that the differences noted from Day 0 to 14 were not as prominent from Day 14 to Day 21.

At Day 35, all pathogens in both groups demonstrated a significant decrease, and there were no significant differences between groups. When comparing the pathogen counts from Day 0 and Day 35, *T. forsythia* counts were slightly higher in the Colgate Great Regular Flavor group, and *T.denticola* were slightly lower when compared to Colgate Total.® However, while the *P. gingivalis* counts in the Colgate Total® group returned to their original Day 0 value, the count in the Colgate Great Regular Flavor group was .40 units higher (Table 16 and Table 37). It appears that the triclosan-copolymer had a continued effect on *P.gingivalis* during the entire study period. This finding is significant because in the study by Socransky and colleagues, periodontal pockets containing *P.gingivalis* exhibited the greatest depth, regardless if *P.gingivalis* was discovered alone or with the other members of the Red Complex.[62]

Analysis of the Grey Complex pathogens revealed all pathogens increased from Day 0 to Day 14, with no significant differences between groups (Table 17). From Day 14 to Day 21, several of the pathogen counts decreased, including *E.saburreum, P.acnes, and S.noxia* (Colgate Great Regular Flavor), *and L.bucallis and N.mucosa* (Colgate Total®); however, these were not significant decreases, and there were no significant differences between groups (Table 24). *S.noxia* was near significance, with the

Colgate Great Regular Flavor dentifrice demonstrating a tendency to inhibit proliferation of this pathogen when compared to Colgate Total® (Table 24).

On Day 35, all Grey Complex pathogens decreased in count, with the exception of *N.mucosa* in the Colgate Total® group, which increased (though not significantly) (Table 38). Analysis of the changes from Day 0 to Day 35 revealed minor differences in counts, with the Colgate Total® group counts lower in six of the ten pathogens, and the Colgate Great Regular Flavor group higher in seven of the ten pathogens at Day 35 (Table 17 and Table 38). The only notable difference was the increase of *N.mucosa* counts in the Colgate Total® group, which was 1.54 units higher on Day 35 than Day 0 (Table 17 and 38).

Based on the findings of this study**,** it is apparent that the antimicrobial effect of triclosan-copolymer is not more effective at reducing gingival inflammation and plaque accumulation. However, Colgate Total® inhibited the proliferation of a limited number of known periodontal pathogens when compared to a standard of care fluoride dentifrice in the absence of mechanical plaque removal. Previous studies of Colgate Total® have conveyed evidence of the antimicrobial and anti-inflammatory effect of this dentifrice on gingivitis reduction, plaque control, and periodontal health.[8–12,71,72] However, in this study, brushing and flossing was eliminated to determine the sole effect of triclosan-copolymer formulation on microbial pathogens. The control group was dispensed Colgate Great Regular Flavor toothpaste, which contains the active ingredient sodium monofluorophosphate 0.76% (0.15% w/v fluoride ion), and the inactive ingredients dicalcium phosphate dihydrate, water, glycerin, sorbitol, sodium lauryl sulfate, cellulose gum, flavor, tetrapotassium pyrophosphate, and sodium saccharin.[82] The results of this

study revealed there was no statistical significance between groups for the clinical measures (with the exception of the Day 14 Plaque Index), and the microbial pathogen differences were limited; however, the lack of a true control group fails to eliminate the possibility that one of the ingredients in the Colgate Great Regular dentifrice is also effective at preventing microbial plaque formation and gingivitis. Colgate Great Regular Flavor contains sodium fluoride, and research has shown that sodium fluoride use has been effective at reducing microbial pathogen load.[87]

The results of this study contribute to the current body of knowledge because it appears that the triclosan-copolymer dentifrice has an effect on known periodontal pathogens such as those in the Orange Complex group, *P.gingivalis,* and *A.actinomycetemcomitans* in the absence of mechanical plaque removal.

There were several limitations in the research design that could have affected the outcome. The time commitment required for participants on Day 14 and Day 21 may have narrowed the pool of eligible candidates due to work, school, or familial obligations. The extensive inclusion and exclusion criteria also limited the number of qualified subjects, which was evidenced through the high percentage of individuals who failed the initial screening appointment (49 percent). To be eligible for randomization into the study, a Probing Pocket Depth of $\leq$ 4 mm in all sites, a Mean Clinical Attachment Level $\leq$ 2 mm on each tooth, and a BOP score of $\leq$ to ten percent at Baseline was mandatory. Because of the high prevalence of gingivitis in adults, the results from the study participants may not be representative of the entire population. In addition, the composition of plaque during an experimental gingivitis model differs from traditional gingivitis, which further limits the ability to generalize the results to the

population. Furthermore, the triclosan copolymer formulation may have been ineffective on such exceptionally healthy gingiva, and the three week timeframe of the experimental gingivitis model might not have been sufficient for the anti-inflammatory properties of Colgate Total® to manifest.

The lack of a sample size calculator cannot ensure that there was a large enough sample for adequate statistical power.[85] Although trends were observed revealing the ability of the triclosan – copolymer formulation to inhibit the proliferation of certain known periodontal pathogens, the small sample size may have prohibited the detection of a statistical difference. Furthermore, the protocol for placement and removal of the stent for the daily brushings created the possibility that the isolated plaque biofilm overgrowth may have become dislodged, which could have affected both the Gingival and Plaque Indices, and the total microbial quantity.

A true control group that did not use any toothpaste in the stent was not included as a component of the study protocol, so there is no data to compare a true control with the other two dentifrices. Moreover, all subjects were dispensed Colgate Great Regular Flavor dentifrice for use during the "washout" period (Day -14 to Baseline), and resumed use of this dentifrice during the recovery phase (Day 21 to Day 35). It is possible that the control group's use of the same dentifrice for the entire 49 day study may have affected the outcome, because the oral flora were already accustomed to the ingredients in the Colgate Great Regular Flavor dentifrice.

Although all thirty subjects demonstrated compliance based on the weight of toothpaste remaining (less than 60 percent), two subjects in the Colgate Total group® were close to non-complaint, returning 54 and 55 percent of their toothpaste (Table 39).

The highest percentage of toothpaste returned in the Colgate Great Regular Flavor group was 41 (Table 39). The combined average toothpaste returned of both groups was 27 percent; however, the Colgate Total® group average was 30.25 percent, and the Colgate Great Regular Flavor average was 22.64 percent (Table 39). The use of more toothpaste by the Colgate Great Regular Flavor group may have impacted the study outcomes.

Although all examiners were calibrated, and every effort was made for the same examiner to evaluate the same subjects over the 49 day study period, there were instances that this was not possible due to the patient's schedules and the study protocol; therefore, intra-examiner reliability could have affected the significant difference in plaque accumulation between groups on the Day 14 study visit.

5.3 Future Directions

The effect of Colgate Total's® triclosan-copolymer formulation on the development of gingivitis has been extensively studied using clinical parameter such as bleeding on probing, the Gingival and Plaque Indices, oral fluids such as saliva and gingival crevicular fluid, and microbial pathogens. Although the results of this study revealed limited differences in regard to the 40 microbial pathogens analyzed, the known periodontal pathogens that did exhibit differences warrant further study on this topic. The preponderance of patients incompliant with oral hygiene recommendations combined with those with limited dexterity (such as the elderly, arthritic, and developmentally disabled) compels the need for evidence-based product recommendations that can improve their oral health.

New directions should also be considered for future research studies. The harvesting and analysis of gingival tissue samples could be conducted to determine the effect of Colgate Total® toothpaste on gingival tissue morphology and transcriptomes. Offenbacher and colleagues used a stent for isolation in an experimental gingivitis model to evaluate the change in gene-expression profiles in tissue samples during the 35 day time period.[88] A study by Jönsson and colleagues evaluated the Gingival Index, microbial pathogens (using the checkerboard DNA-DNA hybridization method previously described by Socransky), and harvested tissue samples to compare differences in gene expression at all study time points during an experimental gingivitis model.[89] Similar studies could be conducted to compare the effect of Colgate Total® toothpaste (isolated in a stent) and a true control group (with an empty stent) to determine the effect of this dentifrice on gene expression in tissue samples. A recent study by Lee et al was able to classify participants who abstained from oral hygiene as either high or low responders based on their inflammatory response, and the clinical and microbial changes were similar to previous experimental gingivitis studies.[54] Similarly, an experimental gingivitis study with Colgate Total® toothpaste could be conducted to determine the effect of this dentifrice on microbial pathogens in subjects classified as having a high response to gingivitis.

CHAPTER VI

CONCLUSIONS

The purpose of this study was to determine the effect of Colgate Total® toothpaste (compared to a standard of care fluoride dentifrice) on microbial pathogens in plaque biofilm samples during an experimental gingivitis model in the absence of mechanical plaque removal. Oral inflammatory disease is widespread, and the relationship between oral health and systemic disease affirms the need to maintain proper oral health.[1,5] The rationale for this research study was to ascertain if the triclosan-copolymer formulation in Colgate Total® could benefit patients who are incompliant with oral hygiene recommendations, and those with compromised manual dexterity such as the elderly, arthritic, or the developmentally disabled through improved oral health.

This research study was conducted during a randomized, controlled, clinical trial at the University of Michigan Center for Oral Health Research (MCOHR). This was a pilot study, with the goal of acquiring data to support a future study on a larger scale. The total number of subjects enrolled for participation was thirty, with fifteen subjects in each arm of the study.

The experimental gingivitis model is often used in clinical research to determine the changes in the inflammatory response as the process of moving from gingival health to

gingivitis occurs in a controlled environment.[7] The statistical analysis included descriptive statistics, independent samples t-tests, paired t-tests, Wilcoxon Signed Rank tests, Wilcoxon Rank Sum tests, and a linear mixed model to control all of the variables in one model. The analysis of the data revealed no significant differences between groups in regard to the Gingival and Plaque Indices and the four microbial pathogens quantities at any time point, with the exception of Day 14,where the Colgate Total® group demonstrated significantly more plaque accumulation compared to the Colgate Great Regular Flavor group (Table 3) (Figure 4.3). Further analysis of the 40 microbial pathogens revealed limited significant differences between groups in regard to known periodontal pathogens such as those in the Orange Complex, *P.gingivalis*, and *A.actinomycetemcomitans*. Limited differences were also seen in the Actinos (Blue Complex) group from Day 0 to Day 14 (Table 11). These results warrant further study on this topic.

Although the examiners and subjects were both blinded to the assignment of study arm, there were multiple examiners involved in data collection. Because the Gingival and Plaque Indices are subjective, it is possible that this data from this statistically significant time point is not truly different. A single calibrated examiner could improve the reliability of the clinical measures such as the Gingival and Plaque indices in future studies. In addition, the use of a true control group could provide additional insight on the effect of Colgate Total® and Colgate Great Regular Favor group on gingival inflammation, plaque accumulation, and the quantity of microbial pathogens associated with gingivitis in oral plaque samples. Furthermore, it is possible that a true statistical difference existed and was not detected because of the small sample size of thirty

subjects. A sample size calculator could determine if the sample size is adequate for the necessary statistical power. Finally, the short duration of the experimental gingivitis model may not have been long enough to exhibit changes in subjects with such healthy gingiva.

Future studies should include new methods such as the harvesting and analysis of gingival tissue samples to determine the effect of Colgate Total® toothpaste on gingival tissue morphology and transcriptomes, and if subjects that classify as high or low responders to gingival inflammation benefit from the triclosan-copolymer formulation or other dentifrices. The aim of future studies should be to provide dental hygienists with the ability to make evidence-based product recommendations to improve the oral health of patients with limited manual dexterity or inadequate oral hygiene.

FIGURES

FIGURE 4.1

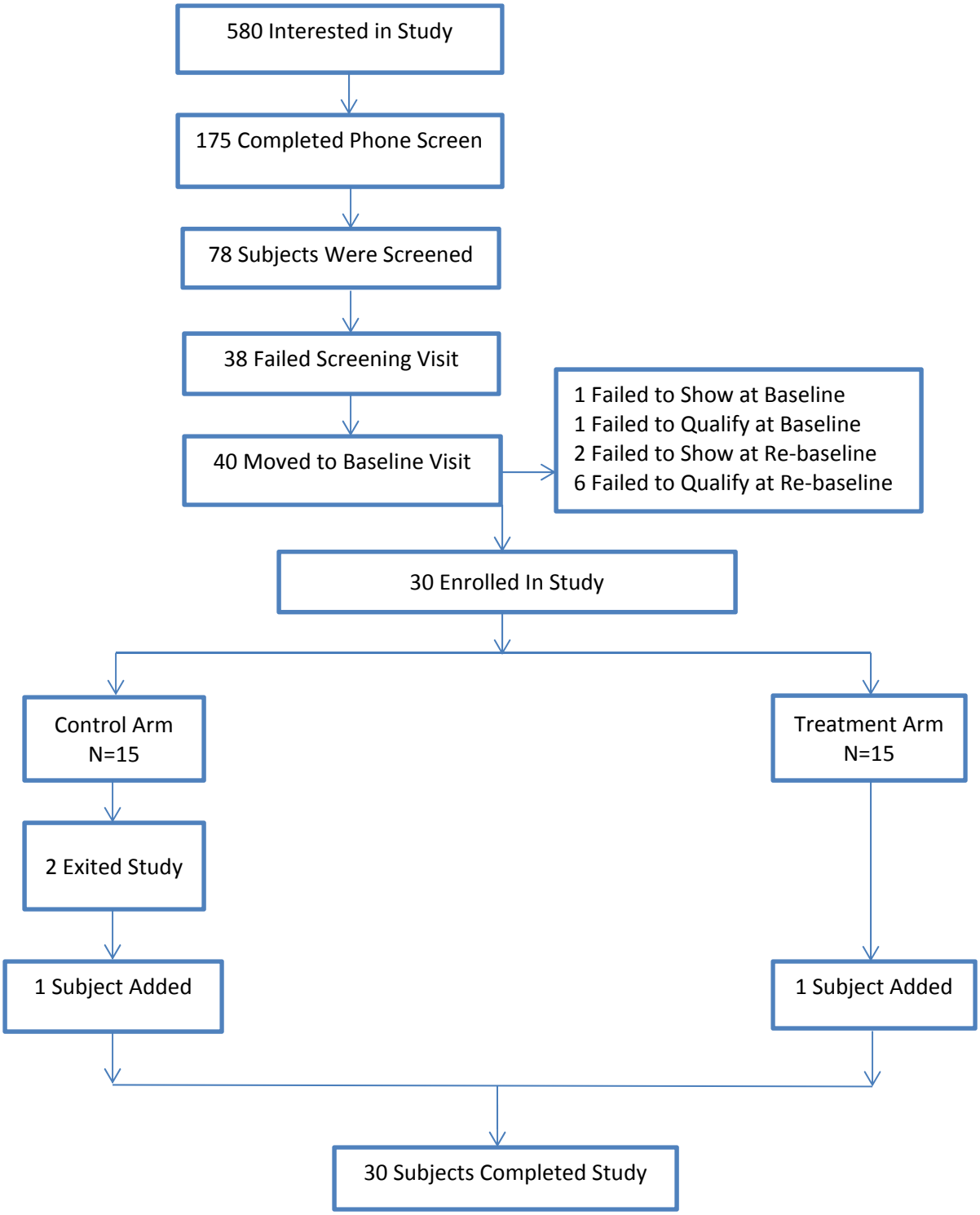

Figure 4.1: Patient Recruitment and Enrollment Chart

FIGURE 4.2

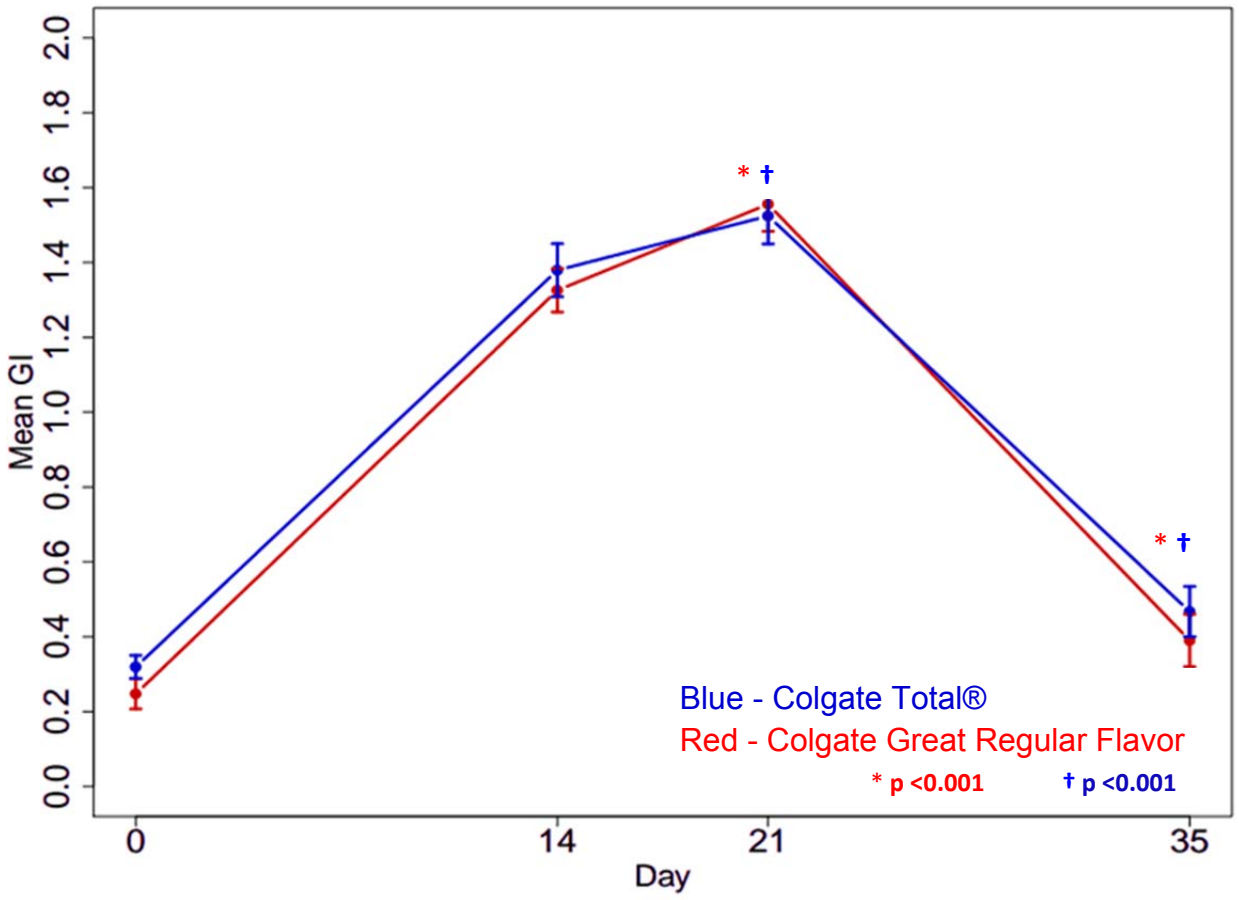

Figure 4.2: Longitudinal Plot of the Gingival Index for the Colgate Total® and Colgate Great Regular Flavor groups during the experimental gingivitis model. No significant differences in the mean GI scores were observed between the two groups.

FIGURE 4.3

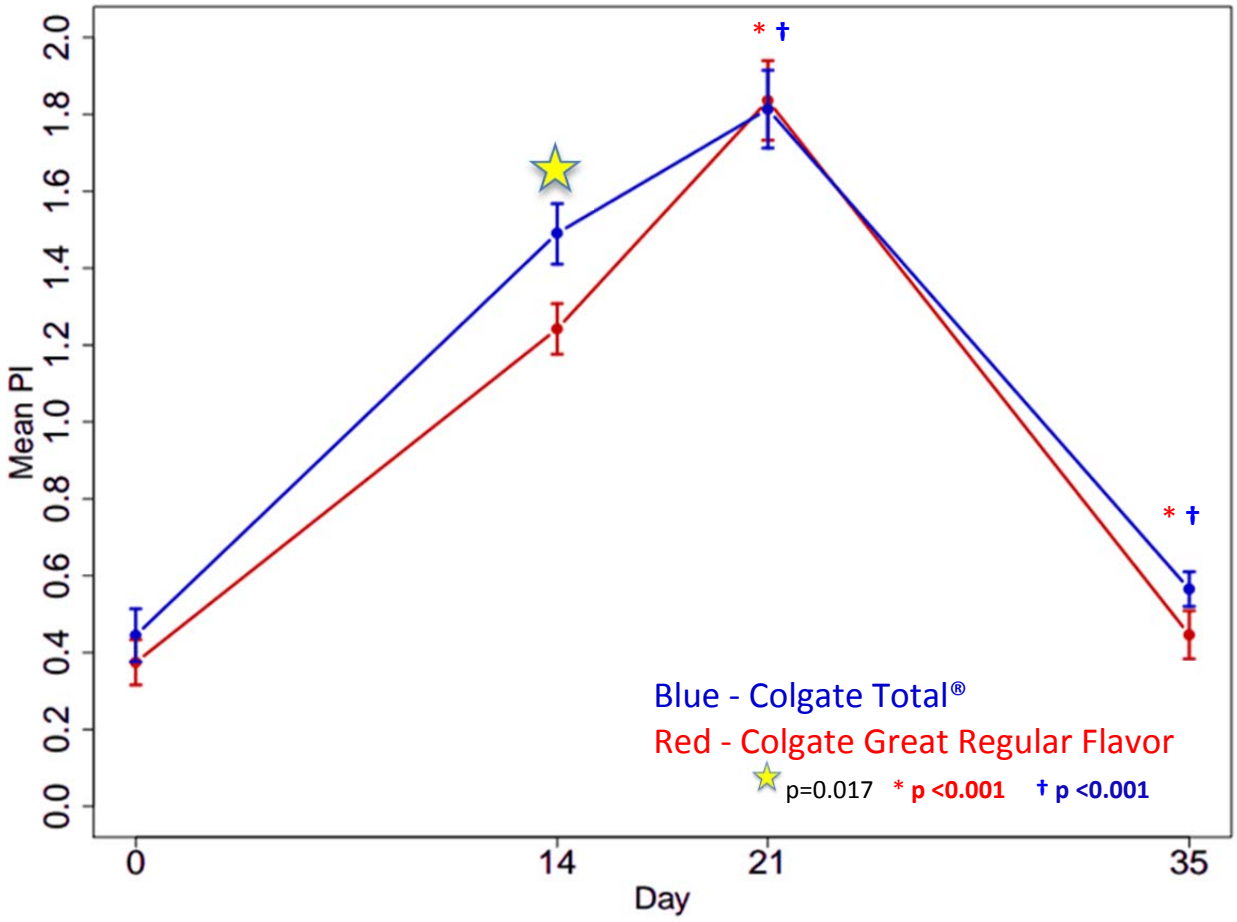

Figure 4.3: Longitudinal Plot of the Plaque Index for both the Colgate Total® and Colgate Great Regular Flavor groups during the experimental gingivitis model. Significant differences in mean plaque scores were observed between groups at Day 14 with the Colgate Total® group having significantly more plaque than the Colgate Great Regular Flavor group.

FIGURE 4.4

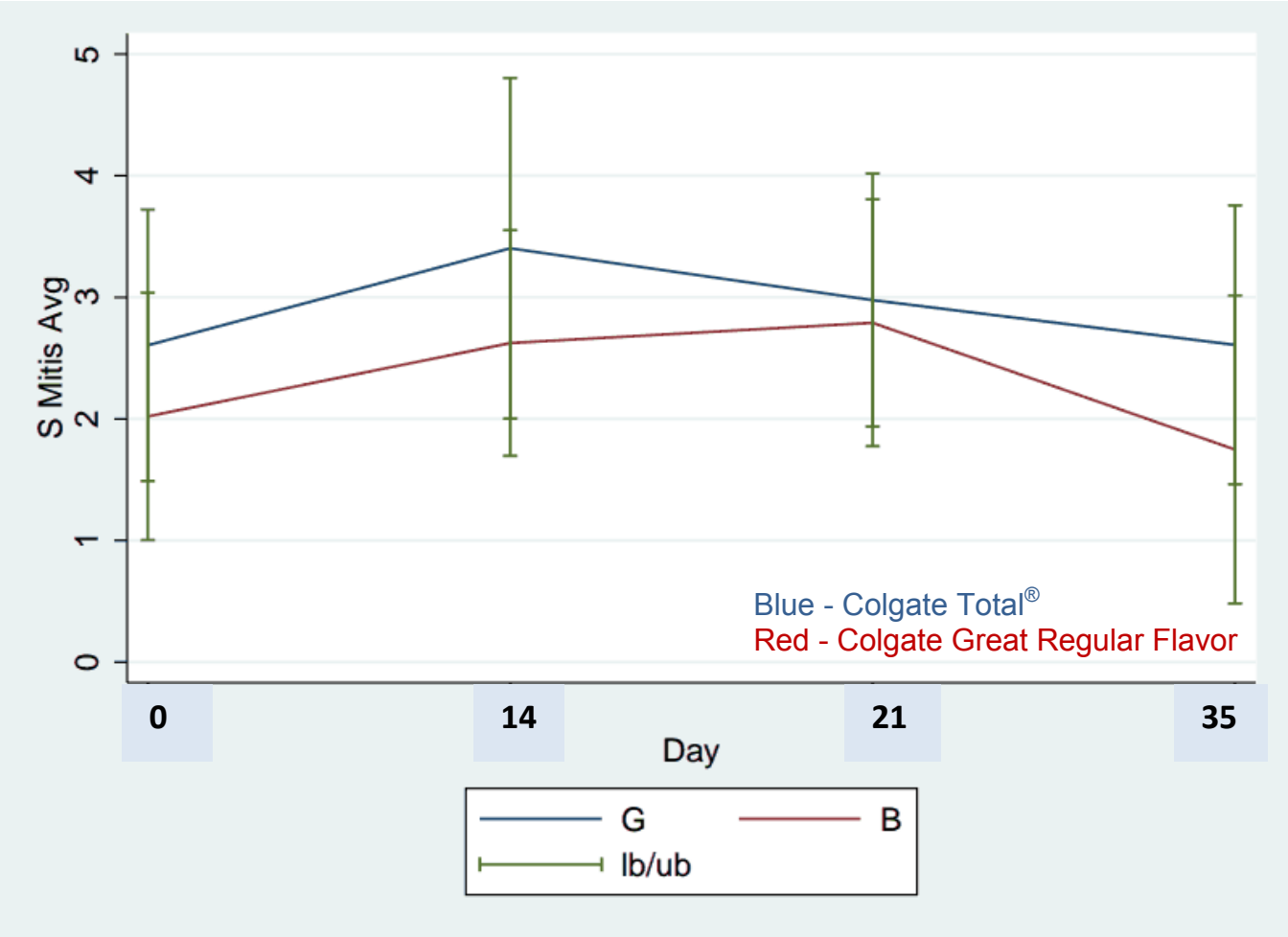

Figure 4.4: Longitudinal Plot of the *S. Mitis* mean pathogen average during the experimental gingivitis model. No significant differences in the mean *S. Mitis* quantities were noted between groups at any time point.

FIGURE 4.5

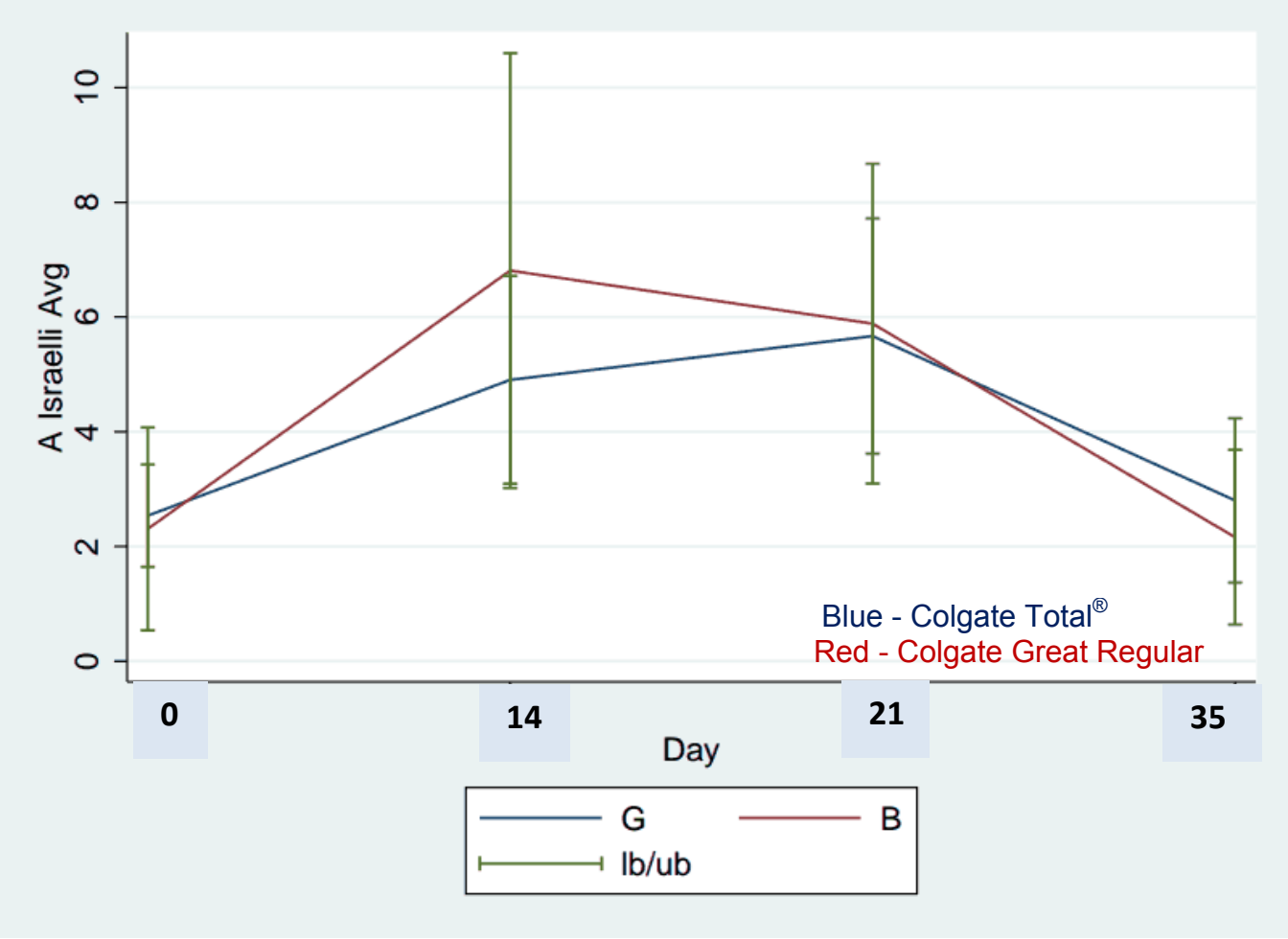

Figure 4.5: Longitudinal Plot of the *A. israelii* mean pathogen average during the experimental gingivitis model. No significant differences in the mean *A. israelii* quantities were noted between groups at any time point.

FIGURE 4.6

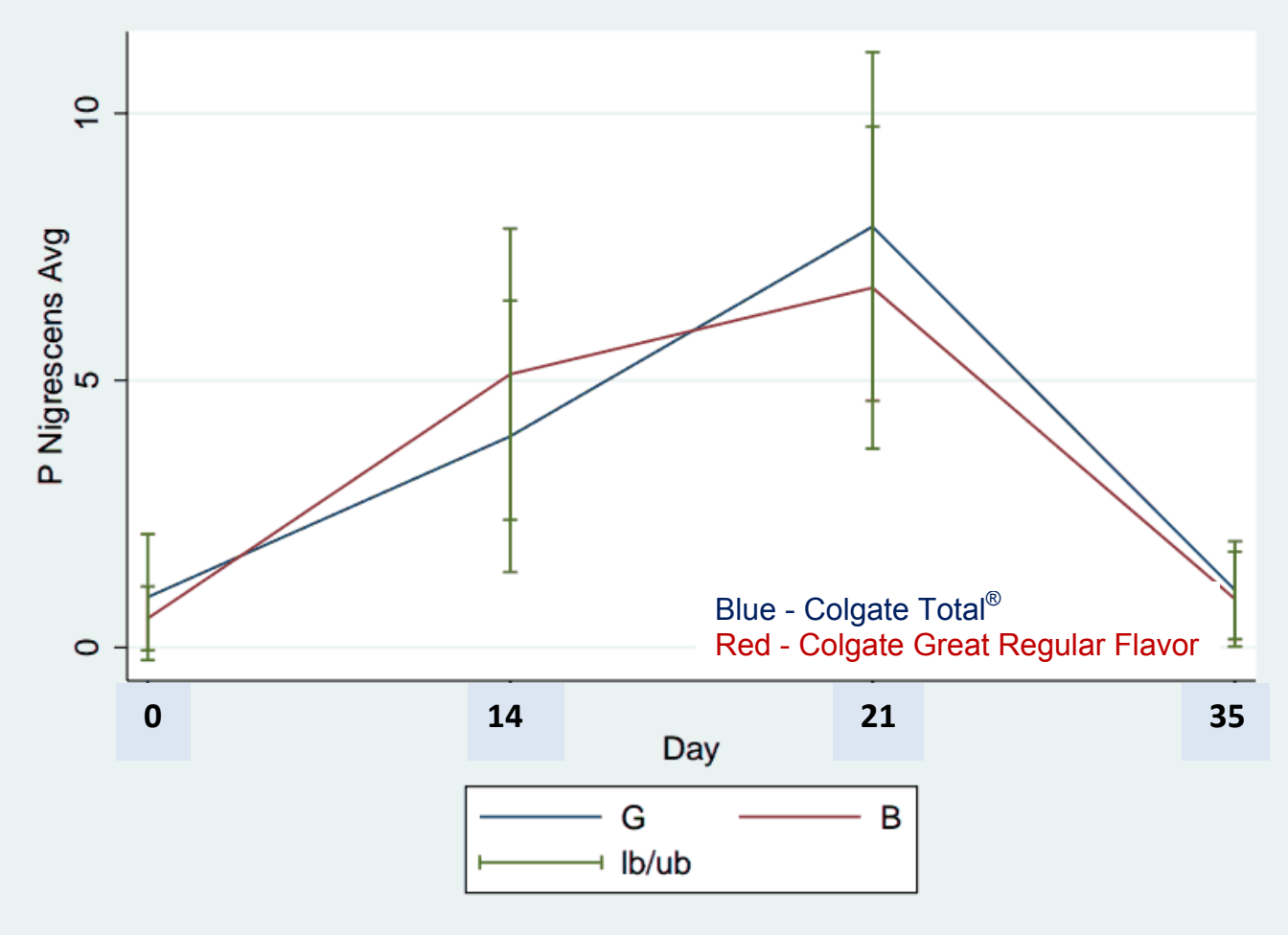

Figure 4.6: Longitudinal Plot of the *P. nigrescens* mean pathogen average during the experimental gingivitis model. No significant differences in the mean *P. nigrescens* quantities were noted between groups at any time point.

FIGURE 4.7

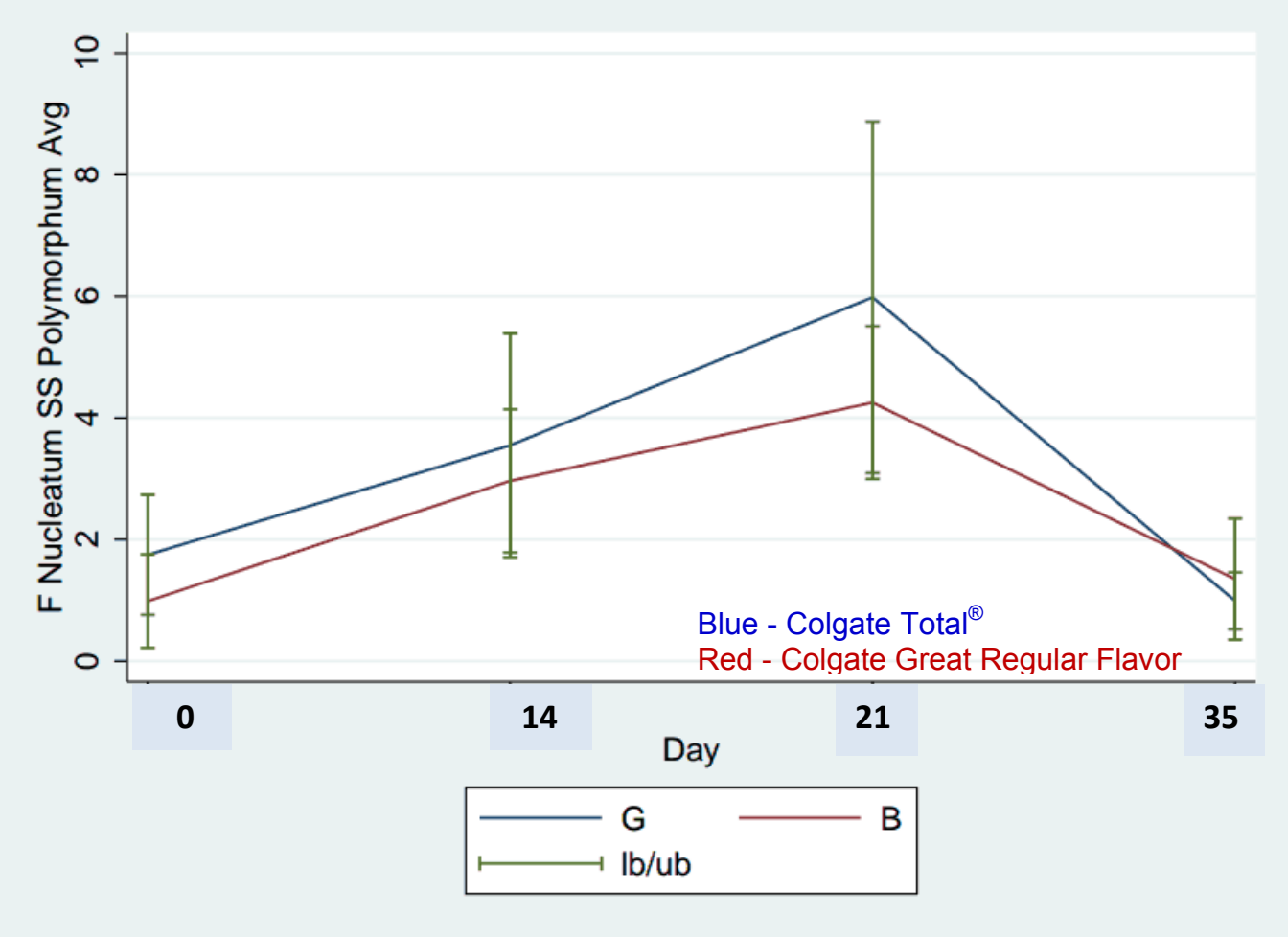

Figure 4.7: Longitudinal Plot of the *F. nucleatum ss polymorphum* mean pathogen Average during the experimental gingivitis model. No significant differences in the mean *F. nucleatum ss polymorphum* quantities were noted between groups at any time point.

FIGURE 4.8

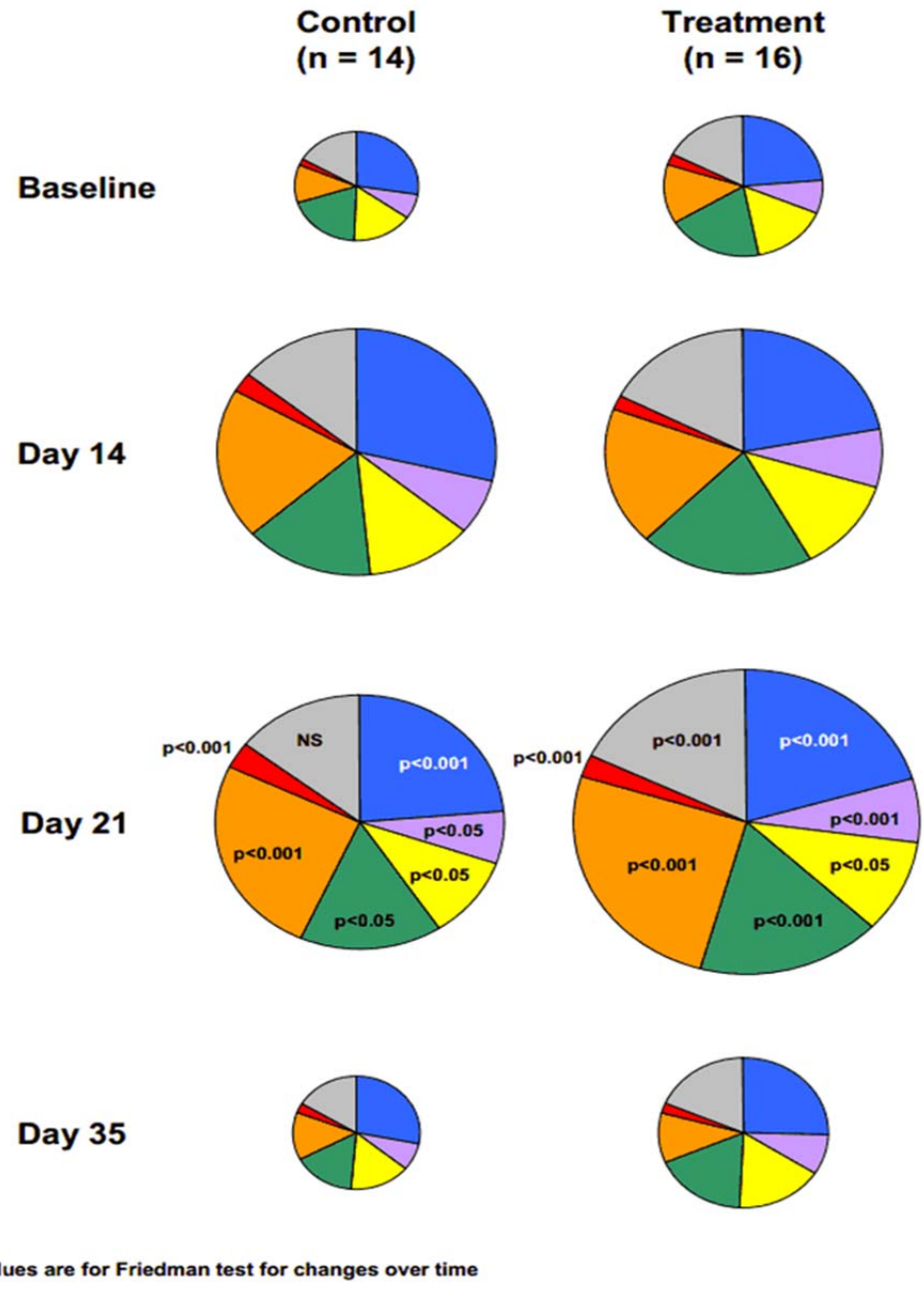

Figure 4.8: Proportions of each bacterial complex as defined by Socransky shown by group over time. Statistically significant differences in quantities of bacteria within each group were observed between Day 0 and Day 21.

FIGURE 4.9

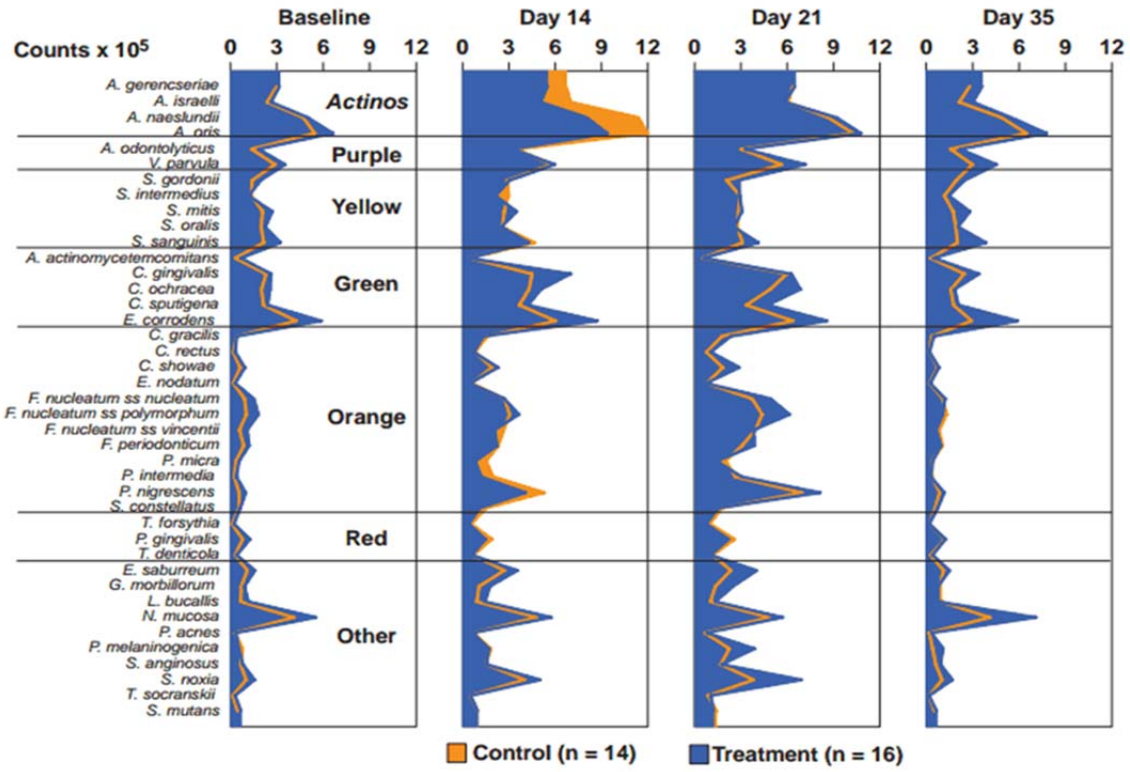

Figure 4.9: Mean quantities of each pathogen stratified by group over time.

TABLES

TABLE 1

Characteristics of Study Participants

	Colgate Total®	Colgate Great Regular Flavor	
	Frequency (%)	Frequency (%)	Fisher's Exact Test p
Gender	16 (100%)	14 (100%)	.118
Male	7 (43.8%)	2 (14.3%)	
Female	9 (56.3%)	12 (85.7%)	
Race	16 (100%)	14 (100%)	1.00
African American	1 (6.3%)	1 (7.1%)	
Asian	2 (12.5%)	2 (14.3%)	
Caucasian	11 (68.8%)	9 (64.3%)	
Hispanic	1 (6.3%)	1 (7.1%)	
Other Race	1 (6.3%)	1 (7.1%)	

	Colgate Total®		Colgate Great Regular Flavor			
	N	Mean (SD)	N	Mean (SD)	t (df)	p
Age	16	26.06 (5.23)	14	27.07 (5.15)	-.531 (28)	.600
CAL at Screening (Day -14)	16	0.76 (0.25)	14	0.75 (0.34)	0.87 (28)	.931
BOP at Screening (Day -14)	16	0.47 (0.11)	14	0.49 (0.13)	-.450 (28)	.656
BOP at Baseline (Day 0)	16	0.09 (0.04)	14	0.10 (0.06)	-.497 (28)	.623

TABLE 2

Paired Samples Tests of BOP Scores

Paired Study Visit	N	Mean Diff. (SD)	t (df)	p
Day -14 to Day 0	30	.42 (.11)	20.37(29)	<.001

Intergroup Paired Samples Tests of BOP Scores

	Colgate Total®				Colgate Great Regular Flavor			
Paired Study Visit	N	Mean Diff. (SD)	t (df)	p	N	Mean Diff. (SD)	t (df)	p
Day -14 to Day 0	16	.41 (.11)	14.58 (15)	<.001	14	.42 (.12)	13.81 (13)	<.001

TABLE 3

Analysis of Gingival Index and Plaque Index

	Colgate Total®		Colgate Great Regular Flavor			
Study Visit	N	Mean (SD)	N	Mean (SD)	t (df)	p
Day 0						
Gingival Index	16	0.32 (0.13)	14	0.24 (0.16)	1.34 (28)	.192
Plaque Index	16	0.44 (0.29)	14	0.38 (0.26)	.607 (28)	.550
Day 14						
Gingival Index	16	1.40 (0.29)	14	1.30 (0.25)	.906 (28)	.373
Plaque Index	16	1.51 (0.31)	14	1.19 (0.26)	3.02 (28)	.005
Day 21						
Gingival Index	16	1.54 (0.30)	14	1.53 (0.30)	.103 (28)	.919
Plaque Index	16	1.85 (0.43)	14	1.76 (0.41)	.526 (28)	.603
Day 35						
Gingival Index	16	0.48 (0.27)	14	0.40 (0.28)	.739 (28)	.466
Plaque Index	16	0.56 (0.19)	14	0.44 (0.24)	1.52 (28)	.140

TABLE 4

Paired Samples Tests of the Gingival Index and Plaque Index for Both Groups Over Time

Paired Study Visit	N	Mean Diff.(SD)	t (df)	p
Day 0 – Day 14				
Gingival Index	30	1.06 (.13)	45.77 (29)	<.001
Plaque Index	30	0.95 (.43)	11.99 (29)	<.001
Day 0 – Day 21				
Gingival Index	30	1.25 (.16)	44.13 (29)	<.001
Plaque Index	30	1.40 (.53)	14.39 (29)	<.001
Day 0 – Day 35				
Gingival Index	30	.157 (.14)	6.27 (29)	<.001
Plaque Index	30	.095 (.30)	1.75 (29)	.091
Day 14 – 21				
Gingival Index	30	.187 (.07)	14.56 (29)	<.001
Plaque Index	30	.445 (.51)	4.83 (29)	<.001
Day 21 – 35				
Gingival Index	30	1. 09 (.06)	98.63 (29)	<.001
Plaque Index	30	1.30 (.44)	16. 30 (29)	<.001

TABLE 5

Intergroup Paired Samples Tests of Gingival and Plaque Index Over Time

	Colgate Total®				Colgate Great Regular Flavor			
Paired Study Visit	N	Mean Diff. (SD)	t (df)	p	N	Mean Diff.(SD)	t (df)	p
Day 0 – Day 14								
Gingival Index	16	1.09 (.09)	46.94 (15)	<.001	14	1.03 (.16)	24.84 (13)	<.001
Plaque Index	16	1.02 (.43)	9.48 (15)	<.001	14	0.87 (.44)	7.39 (13)	<.001
Day 0 – Day 21								
Gingival Index	16	1.27 (.15)	33.87 (15)	<.001	14	1.23 (.16)	28.18 (13)	<.001
Plaque Index	16	1.42 (.58)	9.80 (15)	<.001	14	1.37 (.49)	10.40 (13)	<.001
Day 0 – Day 35								
Gingival Index	16	0.17 (.12)	5.47 (15)	<.001	14	0.15 (.16)	3.49 (13)	.004
Plaque Index	16	0.12 (.34)	1.39 (15)	.186	14	0.07 (.25)	1.03 (13)	.323
Day 14 – Day 21								
Gingival Index	16	0.18 (.07)	10.27 (15)	<.001	14	0.19 (.07)	10.05 (13)	<.001
Plaque Index	16	0.40 (.57)	2.76 (15)	.014	14	0.50 (.43)	4.38 (13)	.001
Day 21 – Day 35								
Gingival Index	16	1.11(.07)	65.03 (15)	<.001	14	1.08 (.05)	80.32 (13)	<.001
Plaque Index	16	1.30 (.46)	11.30 (15)	<.001	14	1.30 (.43)	11.42 (13)	<.001

TABLE 6

Linear Mixed Model for Plaque Index

Variable	Coef.	Std. Error	t (df)	p
Intercept	0.480	0.064	7.48(89.89)	.000
Arm: Control	-0.146	0.065	-2.27(28)	.031
Arm: Treatment	Ref	Ref	Ref	Ref
Day 0	Ref	Ref	Ref	Ref
Day 14	0.951	0.076	12.4(87)	.000
Day 21	1.396	0.076	18.3 (87)	.000
Day 35	0.095	0.076	1.25 (87)	.216

	Variance	Std. Error	p
Residual	0.087	0.013	.000
Intercept	0.009	0.009	.304

Linear Mixed Model for Gingival Index

Variable	Coef..	Std. Error	t (df)	p
Intercept	0.312	0.053	5.83(80.39)	.000
Arm: Control	-0.062	0.057	-1.09(28)	.283
Arm: Treatment	Ref	Ref	Ref	Ref
Day 0	Ref	Ref	Ref	Ref
Day 14	1.064	0.060	17.67(87)	.000
Day 21	1.251	0.060	20.79(87)	.000
Day 35	0.157	0.060	2.62(87)	.011

	Variance	Std. Error	p
Residual	0.054	0.008	.000
Intercept	0.010	0.007	.121

TABLE 7

Analysis of Microbial Pathogens Over Time

	Colgate Total®		Colgate Great Regular Flavor			
Microbial Pathogen	N	Mean (SD)	N	Mean (SD)	t (df)	p
S. mitis						
Day 0	16	2.61 (2.09)	14	2.02 (1.76)	-.821 (28)	.419
Day 14	16	3.40 (2.63)	14	2.62 (1.61)	-.962 (28)	.344
Day 21	16	2.98 (1.95)	14	2.79 (1.76)	-.274 (28)	.786
Day 35	16	2.72 (2.26)	14	1.69 (2.03)	-1.307 (28)	.202
A. israelii						
Day 0	16	2.54 (1.67)	14	2.31 (3.06)	-.261 (28)	.796
Day 14	16	4.90 (3.40)	14	6.81 (6.57)	1.017 (28)	.318
Day 21	16	5.67 (3.85)	14	5.89 (4.83)	.137 (28)	.892
Day 35	16	2.92 (2.83)	14	2.07 (2.44)	-.868 (28)	.393
P. nigrescens						
Day 0	16	0.94 (2.21)	14	0.54 (1.03)	-.617 (28)	.542
Day 14	16	3.95 (4.77)	14	5.12 (4.73)	.669 (28)	.509
Day 21	16	7.88 (6.13)	14	6.74 (5.22)	-.547 (28)	.589
Day 35	16	1.13 (1.82)	14	0.85 (1.43)	-.477 (28)	.637
F. nucleatum ss polymorphum						
Day 0	16	1.75 (1.85)	14	0.99 (1.33)	-1.277 (28)	.212
Day 14	16	3.55 (3.45)	14	2.96 (2.04)	-.554 (28)	.584
Day 21	16	5.98 (5.42)	14	4.25 (2.18)	-1.117 (28)	.274
Day 35	16	1.04 (0.92)	14	1.27 (1.61)	.508 (28)	.615

TABLE 8

Paired Samples Tests of Microbial Pathogens Over Time

Paired Study Visit	N	Mean Diff. (SD)	t (df)	p
Day 0 - Day 14				
S. mitis	30	.71 (1.64)	2.37 (29)	.025
A. israelii	30	3.36 (5.19)	3.55 (29)	.001
P. nigrescens	30	3.74 (4.04)	5.07 (29)	<.001
F. nucleatum ss polymorphum	30	1.88 (2.56)	4.04 (29)	<.001
Day 0 – Day 21				
S. mitis	30	.56 (2.22)	1.38 (29)	.179
A. israelii	30	3.34 (4.28)	4.28 (29)	<.001
P. nigrescens	30	6.59 (5.22)	6.91 (29)	<.001
F. nucleatum ss polymorphum	30	3.78 (3.89)	5.33 (29)	<.001
Day 0 – Day 35				
S. mitis	30	.10 (2.19)	.241 (29)	.811
A. israelii	30	.10 (2.61)	.200 (29)	.843
P. nigrescens	30	.24 (2.35)	.566 (29)	.576
F. nucleatum ss polymorphum	30	.25 (1.94)	.690 (29)	.495
Day 14 – Day 21				
S. mitis	30	.150 (2.18)	.397 (29)	.709
A. israelii	30	.024 (2.95)	.045 (29)	.965
P. nigrescens	30	2.85 (3.66)	.668 (29)	<.001
F. nucleatum ss polymorphum	30	1.90 (2.93)	.535 (29)	.001
Day 21 - Day 35				
S. mitis	30	.654 (2.33)	1.54 (29)	.135
A. israelii	30	3.24 (3.03)	.554 (29)	<.001
P. nigrescens	30	6.35 (5.21)	6.67(29)	<.001
F. nucleatum ss polymorphum	30	4.03 (4.16)	.759 (29)	<.001

TABLE 9

Intergroup Paired Samples Tests of Microbial Pathogens Over Time

	Colgate Total®				Colgate Great Regular Flavor			
Paired Study Visit	N	Mean Diff. (SD)	t (df)	p	N	Mean Diff. (SD)	t (df)	p
Day 0 - Day 14								
S. mitis	16	.80 (1.04)	3.08 (15)	.008	14	.60 (2.17)	1.04 (13)	.317
A. israelii	16	2.37 (3.41)	2.78 (15)	.014	14	4.50 (6.63)	2.54 (13)	.025
P. nigrescens	16	3.01 (3.51)	3.43 (15)	.004	14	4.57 (4.56)	3.76 (13)	.002
F. nucleatum ss polymorphum	16	1.80 (3.08)	2.33 (15)	.034	14	1.98 (1.89)	3.91 (13)	.002
Day 0 – Day 21								
S. mitis	16	.37 (2.22)	.67 (15)	.512	14	.77 (2.28)	1.26 (13)	.229
A. israelii	16	3.13 (3.29)	3.81 (15)	.002	14	3.58 (5.31)	2.52 (13)	.026
P. nigrescens	16	6.94 (5.44)	5.10 (15)	<.001	14	6.19 (5.12)	4.52 (13)	.001
F. nucleatum ss polymorphum	16	4.24 (4.86)	3.49 (15)	.003	14	3.27 (2.42)	5.05 (13)	<.001
Day 0 – Day 35								
S. mitis	16	.11 (2.30)	.19 (15)	.849	14	.33 (2.13)	.59 (13)	.567
A. israelii	16	.38 (2.81)	.543 (15)	.595	14	.23 (2.42)	.36 (13)	.726
P. nigrescens	16	.19 (2.91)	.26 (15)	.796	14	.30 (1.61)	.70 (13)	.495
F. nucleatum ss polymorphum	16	.71 (2.13)	1.33 (15)	.202	14	.29 (1.61)	.67 (13)	.514
Day 14 – Day 21								
S. mitis	16	0.43 (2.64)	.646 (15)	.528	14	.167 (1.53)	.408 (13)	.690
A. israelii	16	0.76 (2.95)	1.04 (15)	.317	14	.925 (2.78)	1.24 (13)	.235
P. nigrescens	16	3.93 (3.94)	3.99 (15)	.001	14	1.62 (2.99)	2.03 (13)	.063
F. nucleatum ss polymorphum	16	2.44 (3.38)	2.88 (15)	.011	14	1.29 (2.28)	2.11 (13)	.055
Day 21 – Day 35								
S. mitis	16	0.26 (1.69)	.616 (15)	.547	14	1.10 (2.89)	1.43 (13)	.177
A. israelii	16	2.74 (2.57)	4.28 (15)	.001	14	3.81 (3.50)	4.07 (13)	.001
P. nigrescens	16	6.75 (5.56)	4.85 (15)	<.001	14	5.89 (4.95)	4.45 (13)	.001
F. nucleatum ss polymorphum	16	4.95 (5.23)	3.78 (15)	.002	14	2.98 (2.18)	5.11 (13)	<.001

TABLE 10

Baseline Microbial Data By Complex

Control Group								
Subject ID	**Blue (*Actinos*)**	**Orange**	**Red**	**Green**	**Purple**	**Yellow**	**Grey (Other)**	**Total**
3	5.19	0.51	0.38	0.67	0.73	3.61	1.13	12.22
4	1.74	0.13	0.26	0.08	0.10	0.28	0.30	2.89
6	4.70	0.23	0.06	0.44	0.31	2.05	0.67	8.44
7	44.66	25.42	2.96	22.79	11.34	20.27	26.22	153.65
10	43.94	10.76	3.37	19.18	8.97	18.71	17.79	122.72
11	1.00	1.11	0.54	1.49	0.65	2.07	2.61	9.47
13	2.45	0.88	0.21	2.18	0.98	4.50	2.45	13.66
15	16.30	10.08	2.13	38.67	3.51	23.41	19.70	113.80
19	4.52	2.37	0.49	1.98	2.70	7.08	2.99	22.13
24	14.54	8.55	1.61	11.07	8.94	15.96	20.15	80.82
25	51.08	18.21	2.08	29.03	13.11	16.84	17.58	147.92
26	6.81	0.54	x	0.32	0.42	0.08	0.80	x
29	14.13	9.60	1.20	21.20	5.72	6.72	21.87	80.43
31	0.01	0.03	0.08	0.04	0.01	0.04	0.16	0.36
Mean	15.08	6.31	1.18	10.65	4.11	8.69	9.60	59.12
Std Dev	4.78	2.11	0.32	3.49	1.24	2.26	2.69	16.42

Test Group								
Patient ID	**Blue**	**Orange**	**Red**	**Green**	**Purple**	**Yellow**	**Grey**	**Total**
1	6.24	4.69	0.57	13.71	1.19	8.83	10.21	45.43
2	17.87	4.66	0.69	7.96	5.83	2.14	5.87	45.01
5	16.84	1.66	0.82	6.37	2.19	2.40	4.44	34.72
8	18.38	5.53	2.51	6.50	3.41	14.26	10.86	61.44
9	23.50	8.76	2.74	13.87	7.25	14.39	14.70	85.20
12	4.38	2.00	0.95	2.55	0.98	4.75	2.52	18.14
14	3.58	0.32	0.23	0.91	0.43	2.69	1.18	9.34
16	8.39	0.27	0.32	0.18	0.88	1.34	0.67	12.05
18	22.34	4.74	1.64	5.91	8.65	8.02	7.38	58.68
20	13.03	21.26	3.70	22.66	5.83	30.78	27.38	124.65
21	13.38	35.06	3.24	23.34	4.28	8.50	20.89	108.68
22	41.39	8.69	2.33	9.63	9.72	27.00	13.80	112.56
27	39.37	25.60	3.55	53.18	11.59	21.22	40.28	194.77
28	7.80	15.07	3.07	29.95	2.94	9.37	26.29	94.49
30	14.09	10.64	1.27	9.70	10.12	12.05	11.02	68.90
32	20.19	7.17	1.71	13.60	8.01	7.41	10.48	68.56
Mean	16.92	9.76	1.83	13.75	5.20	10.95	13.00	71.41
Std Dev	2.76	2.47	0.30	3.35	0.92	2.20	2.72	12.09

Comparison Between Groups								
***p*-value**	0.73	0.31	0.15	0.53	0.48	0.48	0.38	0.54

TABLE 11

Changes in Actinos (Blue) Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
A.gerencseriae	Control	2.92	6.51	3.59	**0.009**
	Test	3.08	5.35	2.27	**0.039**
	p-value *			0.448	
A.israelli	Control	2.31	6.81	4.50	**0.002**
	Test	2.54	4.90	2.37	**0.008**
	p-value *			0.552	
A.naeslundi	Control	4.57	11.05	6.48	**0.000**
	Test	4.87	7.80	2.93	**0.044**
	p-value *			**0.093**	
A.oris	Control	5.29	11.66	6.38	**0.004**
	Test	6.44	9.09	2.65	**0.044**
	p-value *			**0.142**	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 12

Changes in Yellow Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
S.gordonii	Control	1.36	2.82	1.46	**0.002**
	Test	1.90	2.96	1.05	**0.006**
	p-value *			0.951	
S.intermedia	Control	1.30	2.88	1.58	**0.025**
	Test	1.14	2.07	0.93	**0.003**
	p-value *			0.400	
S.mitis	Control	2.02	2.62	0.60	0.268
	Test	2.60	3.40	0.80	**0.011**
	p-value *			0.580	
S.oralis	Control	1.92	2.51	0.59	0.502
	Test	2.19	2.44	0.25	0.348
	p-value *			0.886	
S.sanguinis	Control	2.09	4.51	2.42	**0.002**
	Test	3.11	4.15	1.04	**0.034**
	p-value *			0.208	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 13

Changes in Purple Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
A.odontolyticus	Control	1.31	3.43	2.11	**0.001**
	Test	1.78	3.13	1.36	**0.003**
	p-value *			0.271	
V.parvula	Control	2.79	5.60	2.80	**0.035**
	Test	3.42	5.81	2.39	**0.034**
	p-value *			0.552	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 14

Changes in Green Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
A.actinomycetemcomitans	Control	0.29	0.44	0.15	0.153
	Test	0.67	0.43	-0.24	0.144
	p-value *			**0.012**	
C.ochracea	Control	1.93	4.17	2.24	**0.035**
	Test	2.47	4.94	2.47	**0.034**
	p-value *			0.854	
C.gingivalis	Control	2.24	4.36	2.12	**0.030**
	Test	2.57	6.80	4.23	**0.025**
	p-value *			0.608	
C.sputige	Control	2.08	3.57	1.49	0.078
	Test	2.34	4.14	1.80	**0.039**
	p-value *			0.637	
E.corrodens	Control	4.11	5.86	1.74	0.326
	Test	5.70	8.47	2.77	0.252
	p-value *			0.951	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 15

Changes in Orange Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
C.gracilis	Control	0.40	1.55	1.14	**0.000**
	Test	0.40	1.31	0.91	**0.001**
	p-value *			0.608	
C.rectus	Control	0.19	0.78	0.59	**0.005**
	Test	0.29	0.72	0.42	**0.009**
	p-value *			0.822	
C.showae	Control	0.60	1.84	1.23	**0.009**
	Test	0.90	2.23	1.33	**0.009**
	p-value *			0.790	
E.nodatum	Control	0.19	0.60	0.40	**0.003**
	Test	0.29	0.47	0.18	**0.006**
	p-value *			0.289	
F.nucleatum.ss.nucleatum	Control	0.87	2.66	1.79	**0.000**
	Test	1.46	2.61	1.15	**0.011**
	p-value *			0.131	
F.nucleatum.ss.polymorphum	Control	0.99	2.96	1.98	**0.001**
	Test	1.75	3.55	1.80	**0.009**
	p-value *			0.334	
F.nucleatum.ss.vincentii	Control	0.57	2.59	2.02	**0.001**
	Test	1.05	2.01	0.96	**0.009**
	p-value *			0.154	
F.periodonticum	Control	0.82	2.26	1.44	**0.000**
	Test	1.18	2.17	0.99	**0.002**
	p-value *			0.275	
P.micra	Control	0.35	1.47	1.12	**0.001**
	Test	0.53	0.82	0.29	**0.039**
	p-value *			0.077	
P.intermedia	Control	0.24	1.93	1.68	**0.001**
	Test	0.36	1.18	0.82	**0.002**
	p-value *			0.224	
P.nigrescens	Control	0.54	5.12	4.57	**0.000**
	Test	0.94	3.95	3.01	**0.000**
	p-value *			0.423	
S.constellatus	Control	0.54	1.50	0.95	**0.005**
	Test	0.61	1.07	0.46	**0.004**
	p-value *			0.240	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 16

Changes in Red Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
T.forsythia	Control	0.11	0.54	0.43	**0.001**
	Test	0.23	0.41	0.18	**0.001**
	p-value *			0.131	
P.gingivalis	Control	0.74	1.84	1.10	**0.000**
	Test	1.22	1.44	0.22	0.193
	p-value *			**0.006**	
T.denticola	Control	0.30	0.66	0.37	**0.002**
	Test	0.38	0.59	0.20	**0.000**
	p-value *			0.275	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 17

Changes in Other (Grey) Complex Microbes from Day 0 to Day 14 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 14	Change from Day 0 to Day 14	*p-value***
E.saburreum	Control	1.06	2.62	1.56	**0.013**
	Test	1.54	3.45	1.91	**0.001**
	p-value *			0.984	
G.morbillorum	Control	0.69	1.06	0.37	0.078
	Test	0.88	1.72	0.84	**0.016**
	p-value *			0.822	
L.bucallis	Control	0.62	0.90	0.28	0.058
	Test	1.12	1.40	0.28	0.323
	p-value *			0.294	
N.mucosa	Control	3.99	4.67	0.67	0.761
	Test	5.35	5.59	0.24	0.860
	p-value *			0.886	
P.acnes	Control	0.29	0.70	0.41	**0.005**
	Test	0.34	0.65	0.32	**0.000**
	p-value *			0.822	
P.melaninogenica	Control	0.77	1.78	1.01	**0.013**
	Test	0.51	1.64	1.13	**0.000**
	p-value *			0.697	
S.anginosus	Control	0.63	1.48	0.85	**0.007**
	Test	0.78	1.52	0.73	**0.000**
	p-value *			0.951	
S.noxia	Control	0.98	3.90	2.92	**0.000**
	Test	1.50	4.89	3.39	**0.000**
	p-value *			0.608	
T.socranskii	Control	0.15	0.51	0.35	**0.011**
	Test	0.29	0.52	0.23	**0.013**
	p-value *			0.552	
S.mutans	Control	0.43	0.95	0.52	**0.002**
	Test	0.67	0.94	0.27	**0.002**
	p-value *			0.275	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 18

Changes in Actinos (Blue) Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
A.gerencseriae	Control	6.51	6.20	-0.31	0.502
	Test	5.35	6.30	0.95	0.144
	p-value *			0.101	
A.israelli	Control	6.81	5.89	-0.93	0.241
	Test	4.90	5.67	0.76	0.175
	p-value *			0.052	
A.naeslundi	Control	11.05	8.40	-2.65	0.153
	Test	7.80	8.94	1.14	0.105
	p-value *			0.070	
A.oris	Control	11.66	9.97	-1.69	0.194
	Test	9.09	10.46	1.38	0.159
	p-value *			0.077	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 19

Changes in Yellow Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
S.gordonii	Control	2.82	2.02	-0.80	0.068
	Test	2.96	2.83	-0.13	0.433
	p-value *			0.580	
S.intermedia	Control	2.88	2.88	0.01	0.670
	Test	2.07	2.85	0.78	0.464
	p-value *			0.637	
S.mitis	Control	2.62	2.79	0.17	0.761
	Test	3.40	2.98	-0.43	0.669
	p-value *			0.473	
S.oralis	Control	2.51	2.69	0.18	0.583
	Test	2.44	2.49	0.05	0.940
	p-value *			0.697	
S.sanguinis	Control	4.51	3.01	-1.50	**0.035**
	Test	4.15	4.01	-0.13	0.632
	p-value *			0.377	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 20

Changes in Purple Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
A.odontolyticus	Control	3.43	2.95	-0.47	0.217
	Test	3.14	3.21	0.07	0.348
	p-value *			0.120	
V.parvula	Control	5.60	5.45	-0.14	0.502
	Test	5.81	6.96	1.15	0.083
	p-value *			0.110	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 21

Changes in Green Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
A.actinomycetemcomitans	Control	0.44	0.53	0.09	0.135
	Test	0.43	0.56	0.13	0.083
	p-value *			0.759	
C.ochracea	Control	4.17	4.76	0.59	0.391
	Test	4.94	6.65	1.70	0.348
	p-value *			0.886	
C.gingivalis	Control	4.36	5.81	1.45	0.104
	Test	6.80	6.09	-0.71	1.000
	p-value *			0.275	
C.sputige	Control	3.57	3.26	-0.31	0.855
	Test	4.14	4.62	0.49	0.597
	p-value *			0.886	
E.corrodens	Control	5.86	6.18	0.32	0.542
	Test	8.47	8.31	-0.16	0.744
	p-value *			0.552	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 22

Changes in Orange Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
C.gracilis	Control	1.55	1.73	0.18	0.670
	Test	1.31	2.28	0.97	**0.016**
	p-value *			0.208	
C.rectus	Control	0.78	0.77	-0.01	0.626
	Test	0.72	1.04	0.32	0.231
	p-value *			0.790	
C.showae	Control	1.84	1.77	-0.07	0.903
	Test	2.23	2.82	0.59	0.252
	p-value *			0.667	
E.nodatum	Control	0.60	0.76	0.16	0.241
	Test	0.47	0.74	0.27	**0.003**
	p-value *			0.552	
F.nucleatum.ss.nucleatum	Control	2.66	3.64	0.97	**0.045**
	Test	2.61	4.79	2.18	**0.002**
	p-value *			0.271	
F.nucleatum.ss.polymorphum	Control	2.96	4.25	1.29	0.091
	Test	3.55	5.98	2.44	**0.006**
	p-value *			0.637	
F.nucleatum.ss.vincentii	Control	2.59	3.86	1.27	**0.020**
	Test	2.01	3.75	1.74	**0.003**
	p-value *			0.525	
F.periodonticum	Control	2.26	2.98	0.72	0.068
	Test	2.17	3.78	1.61	**0.003**
	p-value *			0.728	
P.micra	Control	1.47	1.96	0.48	0.326
	Test	0.82	1.38	0.57	**0.001**
	p-value *			0.377	
P.intermedia	Control	1.93	2.57	0.64	0.194
	Test	1.18	3.00	1.82	**0.001**
	p-value *			**0.052**	
P.nigrescens	Control	5.12	6.74	1.62	0.078
	Test	3.95	7.88	3.93	**0.001**
	p-value *			**0.034**	
S.constellatus	Control	1.50	1.72	0.23	0.626
	Test	1.07	1.48	0.42	0.375
	p-value *			0.790	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 23

Changes in Red Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
T.forsythia	Control	0.54	0.95	0.41	0.119
	Test	0.41	0.73	0.32	**0.008**
	p-value *			0.608	
P.gingivalis	Control	1.84	2.52	0.68	0.268
	Test	1.44	2.15	0.71	**0.003**
	p-value *			0.193	
T.denticola	Control	0.66	1.16	0.50	0.268
	Test	0.59	1.01	0.42	**0.044**
	p-value *			0.790	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 24

Changes in Other (Grey) Complex Microbes from Day 14 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 14	Day 21	Change from Day 14 to Day 21	*p-value***
E.saburreum	Control	2.62	2.29	-0.33	0.761
	Test	3.45	3.92	0.46	0.323
	p-value *			0.608	
G.morbillorum	Control	1.06	1.34	0.28	0.391
	Test	1.72	2.45	0.72	**0.044**
	p-value *			0.525	
L.bucallis	Control	0.90	1.01	0.11	0.268
	Test	1.40	1.31	-0.09	0.632
	p-value *			0.552	
N.mucosa	Control	4.67	4.69	0.02	0.670
	Test	5.59	5.53	-0.06	0.528
	p-value *			0.854	
P.acnes	Control	0.70	0.67	-0.03	0.761
	Test	0.65	0.80	0.14	0.105
	p-value *			0.448	
P.melaninogenica	Control	1.78	2.17	0.39	0.463
	Test	1.64	3.78	2.14	**0.051**
	p-value *			0.355	
S.anginosus	Control	1.48	1.66	0.18	0.855
	Test	1.52	1.75	0.23	0.464
	p-value *			0.697	
S.noxia	Control	3.90	3.72	-0.19	0.952
	Test	4.89	6.72	1.83	**0.003**
	p-value *			0.085	
T.socranskii	Control	0.51	0.88	0.37	**0.042**
	Test	0.52	1.02	0.50	**0.002**
	p-value *			0.759	
S.mutans	Control	0.95	1.42	0.47	0.391
	Test	0.94	1.21	0.27	0.074
	p-value *			0.637	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 25

Changes in Actinos (Blue) Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
A.gerencseriae	Control	2.92	6.20	3.28	**0.030**
	Test	3.08	6.30	3.22	**0.000**
	p-value *			0.790	
A.israelli	Control	2.31	5.89	3.58	**0.013**
	Test	2.54	5.67	3.13	**0.000**
	p-value *			0.918	
A.naeslundi	Control	4.57	8.40	3.83	**0.011**
	Test	4.87	8.94	4.07	**0.000**
	p-value *			0.951	
A.oris	Control	5.29	9.97	4.69	**0.025**
	Test	6.44	10.46	4.03	**0.001**
	p-value *			0.822	
* via Wilcoxon Rank Sum test ** via Wilcoxon Signed Rank test					

TABLE 26

Changes in Yellow Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
S.gordonii	Control	1.36	2.02	0.66	0.241
	Test	1.90	2.83	0.92	**0.051**
	p-value *			0.951	
S.intermedia	Control	1.30	2.88	1.58	**0.035**
	Test	1.14	2.85	1.71	**0.003**
	p-value *			0.759	
S.mitis	Control	2.02	2.79	0.77	0.241
	Test	2.60	2.98	0.37	0.669
	p-value *			0.608	
S.oralis	Control	1.92	2.69	0.77	0.241
	Test	2.19	2.49	0.30	0.348
	p-value *			0.448	
S.sanguinis	Control	2.09	3.01	0.92	0.426
	Test	3.11	4.01	0.90	0.298
	p-value *			0.790	
* via Wilcoxon Rank Sum test ** via Wilcoxon Signed Rank test					

TABLE 27

Changes in Purple Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
A.odontolyticus	Control	1.31	2.95	1.64	**0.013**
	Test	1.78	3.21	1.43	**0.001**
	p-value *			0.918	
V.parvula	Control	2.79	5.45	2.66	0.135
	Test	3.42	6.96	3.54	**0.002**
	p-value *			0.423	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 28

Changes in Green Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
A.actinomycetemcomitans	Control	0.29	0.53	0.24	0.068
	Test	0.67	0.56	-0.11	0.782
	p-value *			**0.038**	
C.ochracea	Control	1.93	4.76	2.83	**0.011**
	Test	2.47	6.65	4.17	**0.002**
	p-value *			0.759	
C.gingivalis	Control	2.24	5.81	3.57	**0.017**
	Test	2.57	6.09	3.52	**0.001**
	p-value *			0.822	
C.sputige	Control	2.08	3.26	1.18	0.358
	Test	2.34	4.62	2.28	**0.002**
	p-value *			0.854	
E.corrodens	Control	4.11	6.18	2.07	0.296
	Test	5.70	8.31	2.61	0.348
	p-value *			0.608	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 29

Changes in Orange Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
C.gracilis	Control	0.40	1.73	1.33	**0.000**
	Test	0.40	2.28	1.88	**0.000**
	p-value *			0.208	
C.rectus	Control	0.19	0.77	0.58	**0.005**
	Test	0.29	1.04	0.74	**0.000**
	p-value *			0.759	
C.showae	Control	0.60	1.77	1.17	**0.013**
	Test	0.90	2.82	1.92	**0.000**
	p-value *			0.759	
E.nodatum	Control	0.19	0.76	0.56	**0.001**
	Test	0.29	0.74	0.45	**0.000**
	p-value *			0.728	
F.nucleatum.ss.nucleatum	Control	0.87	3.64	2.76	**0.000**
	Test	1.46	4.79	3.33	**0.000**
	p-value *			0.759	
F.nucleatum.ss.polymorphum	Control	0.99	4.25	3.27	**0.001**
	Test	1.75	5.98	4.24	**0.000**
	p-value *			0.984	
F.nucleatum.ss.vincentii	Control	0.57	3.86	3.29	**0.000**
	Test	1.05	3.75	2.70	**0.000**
	p-value *			0.423	
F.periodonticum	Control	0.82	2.98	2.16	**0.001**
	Test	1.18	3.78	2.60	**0.000**
	p-value *			0.525	
P.micra	Control	0.35	1.96	1.60	**0.001**
	Test	0.53	1.38	0.86	**0.003**
	p-value *			0.193	
P.intermedia	Control	0.24	2.57	2.33	**0.000**
	Test	0.36	3.00	2.64	**0.000**
	p-value *			0.918	
P.nigrescens	Control	0.54	6.74	6.19	**0.000**
	Test	0.94	7.88	6.94	**0.000**
	p-value *			0.759	
S.constellatus	Control	0.54	1.72	1.18	**0.005**
	Test	0.61	1.48	0.88	**0.009**
	p-value *			0.498	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 30

Changes in Red Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
T.forsythia	Control	0.11	0.95	0.83	**0.001**
	Test	0.23	0.73	0.50	**0.000**
	p-value *			0.886	
P.gingivalis	Control	0.74	2.52	1.78	**0.002**
	Test	1.22	2.15	0.93	**0.000**
	p-value *			0.714	
T.denticola	Control	0.30	1.16	0.86	**0.005**
	Test	0.38	1.01	0.62	**0.000**
	p-value *			0.918	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 31

Changes in Other (Grey) Complex Microbes from Day 0 to Day 21 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 0	Day 21	Change from Day 0 to Day 21	*p-value***
E.saburreum	Control	1.06	2.29	1.23	**0.020**
	Test	1.54	3.92	2.38	**0.000**
	p-value *			0.759	
G.morbillorum	Control	0.69	1.34	0.65	0.068
	Test	0.88	2.45	1.56	**0.001**
	p-value *			0.400	
L.bucallis	Control	0.62	1.01	0.39	0.068
	Test	1.12	1.31	0.19	0.193
	p-value *			0.275	
N.mucosa	Control	3.99	4.69	0.69	0.670
	Test	5.35	5.53	0.18	0.980
	p-value *			0.552	
P.acnes	Control	0.29	0.67	0.38	**0.007**
	Test	0.34	0.80	0.46	**0.000**
	p-value *			0.728	
P.melaninogenica	Control	0.77	2.17	1.40	**0.007**
	Test	0.51	3.78	3.27	**0.000**
	p-value *			0.608	
S.anginosus	Control	0.63	1.66	1.04	**0.013**
	Test	0.78	1.75	0.96	**0.001**
	p-value *			0.822	
S.noxia	Control	0.98	3.72	2.74	**0.001**
	Test	1.50	6.72	5.22	**0.000**
	p-value *			**0.085**	
T.socranskii	Control	0.15	0.88	0.72	**0.002**
	Test	0.29	1.02	0.73	**0.000**
	p-value *			0.918	
S.mutans	Control	0.43	1.42	0.99	**0.004**
	Test	0.67	1.21	0.54	**0.001**
	p-value *			0.313	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 32

Changes in Actinos (Blue) Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
A.gerencseriae	Control	6.20	2.75	-3.45	**0.000**
	Test	6.30	3.51	-2.79	**0.001**
	p-value *			0.951	
A.israelli	Control	5.89	2.07	-3.81	**0.000**
	Test	5.67	2.92	-2.75	**0.001**
	p-value *			0.552	
A.naeslundi	Control	8.40	4.71	-3.69	**0.013**
	Test	8.94	5.33	-3.61	**0.018**
	p-value *			0.667	
A.oris	Control	9.97	6.29	-3.69	**0.030**
	Test	10.46	7.55	-2.92	**0.029**
	p-value *			0.667	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 33

Changes in Yellow Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
S.gordonii	Control	2.02	1.89	-0.13	0.463
	Test	2.83	2.43	-0.40	0.495
	p-value *			0.728	
S.intermedia	Control	2.88	1.12	-1.77	**0.004**
	Test	2.85	1.44	-1.41	0.058
	p-value *			0.580	
S.mitis	Control	2.79	1.69	-1.10	0.153
	Test	2.98	2.72	-0.26	0.323
	p-value *			0.070	
S.oralis	Control	2.69	1.85	-0.84	0.153
	Test	2.49	2.02	-0.47	0.159
	p-value *			0.240	
S.sanguinis	Control	3.01	1.97	-1.05	0.268
	Test	4.01	3.74	-0.27	0.597
	p-value *			0.697	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 34

Changes in Purple Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
A.odontolyticus	Control	2.95	1.53	-1.43	**0.005**
	Test	3.21	2.13	-1.08	**0.011**
	p-value *			0.984	
V.parvula	Control	5.45	2.89	-2.56	0.173
	Test	6.96	4.40	-2.55	**0.011**
	p-value *			1.000	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 35

Changes in Green Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
A.actinomycetemcomitans	Control	0.53	0.27	-0.26	0.058
	Test	0.56	0.49	-0.08	0.144
	p-value *			**0.179**	
C.ochracea	Control	4.76	1.54	-3.22	**0.002**
	Test	6.65	1.79	-4.86	**0.000**
	p-value *			0.377	
C.gingivalis	Control	5.81	2.40	-3.41	**0.035**
	Test	6.09	3.33	-2.76	0.093
	p-value *			0.580	
C.sputige	Control	3.26	1.71	-1.55	**0.025**
	Test	4.62	2.04	-2.58	**0.005**
	p-value *			1.000	
E.corrodens	Control	6.18	2.86	-3.32	0.078
	Test	8.31	5.74	-2.57	0.231
	p-value *			0.608	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 36

Changes in Orange Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
C.gracilis	Control	1.73	0.33	-1.40	**0.000**
	Test	2.28	0.48	-1.80	**0.000**
	p-value *			0.240	
C.rectus	Control	0.77	0.22	-0.55	**0.005**
	Test	1.04	0.22	-0.81	**0.000**
	p-value *			0.667	
C.showae	Control	1.77	0.72	-1.06	**0.011**
	Test	2.82	0.78	-2.03	**0.000**
	p-value *			0.275	
E.nodatum	Control	0.76	0.21	-0.55	**0.000**
	Test	0.74	0.26	-0.48	**0.000**
	p-value *			0.854	
F.nucleatum.ss.nucleatum	Control	3.64	1.03	-2.61	**0.000**
	Test	4.79	1.18	-3.61	**0.000**
	p-value *			0.637	
F.nucleatum.ss.polymorphum	Control	4.25	1.27	-2.98	**0.000**
	Test	5.98	1.04	-4.95	**0.000**
	p-value *			0.313	
F.nucleatum.ss.vincentii	Control	3.86	0.82	-3.04	**0.000**
	Test	3.75	0.61	-3.14	**0.000**
	p-value *			0.822	
F.periodonticum	Control	2.98	1.00	-1.99	**0.001**
	Test	3.78	0.92	-2.86	**0.000**
	p-value *			0.697	
P.micra	Control	1.96	0.37	-1.59	**0.001**
	Test	1.38	0.41	-0.97	**0.000**
	p-value *			0.525	
P.intermedia	Control	2.57	0.36	-2.21	**0.000**
	Test	3.00	0.30	-2.70	**0.000**
	p-value *			0.697	
P.nigrescens	Control	6.74	0.85	-5.89	**0.000**
	Test	7.88	1.13	-6.75	**0.000**
	p-value *			0.790	
S.constellatus	Control	1.72	0.54	-1.19	**0.007**
	Test	1.48	0.72	-0.76	**0.008**
	p-value *			0.498	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 37

Changes in Red Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
T.forsythia	Control	0.95	0.21	-0.74	**0.001**
	Test	0.73	0.20	-0.54	**0.001**
	p-value *			0.759	
P.gingivalis	Control	2.52	1.14	-1.38	**0.025**
	Test	2.15	1.22	-0.93	**0.003**
	p-value *			0.448	
T.denticola	Control	1.16	0.29	-0.87	**0.007**
	Test	1.01	0.40	-0.61	**0.005**
	p-value *			0.728	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 38

Changes in Other (Grey) Complex Microbes from Day 21 to Day 35 for Dentifrice Groups

		Mean (Log Base 2)			
Pathogen	Group	Day 21	Day 35	Change from Day 21 to Day 35	*p-value***
E.saburreum	Control	2.29	1.07	-1.22	**0.020**
	Test	3.92	1.46	-2.46	**0.002**
	p-value *			0.208	
G.morbillorum	Control	1.34	0.89	-0.45	0.194
	Test	2.45	0.74	-1.71	**0.000**
	p-value *			0.240	
L.bucallis	Control	1.01	0.92	-0.08	0.455
	Test	1.31	0.75	-0.57	**0.003**
	p-value *			0.184	
N.mucosa	Control	4.69	4.01	-0.68	0.391
	Test	5.53	6.89	1.35	0.782
	p-value *			0.498	
P.acnes	Control	0.67	0.20	-0.47	**0.001**
	Test	0.80	0.33	-0.46	**0.000**
	p-value *			1.000	
P.melaninogenica	Control	2.17	0.43	-1.75	**0.001**
	Test	3.78	1.06	-2.72	**0.000**
	p-value *			0.525	
S.anginosus	Control	1.66	0.57	-1.09	**0.017**
	Test	1.75	0.90	-0.85	**0.009**
	p-value *			0.637	
S.noxia	Control	3.72	0.99	-2.73	**0.000**
	Test	6.72	1.64	-5.08	**0.001**
	p-value *			0.120	
T.socranskii	Control	0.88	0.20	-0.68	**0.000**
	Test	1.02	0.20	-0.82	**0.001**
	p-value *			0.728	
S.mutans	Control	1.42	0.49	-0.93	**0.017**
	Test	1.21	0.64	-0.57	**0.016**
	p-value *			0.822	
* via Wilcoxon Rank Sum test		** via Wilcoxon Signed Rank test			

TABLE 39

Subject compliance and dentifrice usage

Subject	Initial Dentifrice Tube weight (grams)	Return Dentifrice Tube weight (grams)	Difference in Dentifrice Tube weight (grams)	Dentifrice Remaining (%)
1	187.71	50.57	137.14	27
2	187.49	41.77	145.72	22
3	195	65.91	129.09	34
4	195.01	59.44	135.57	30
5	187.49	54.37	133.12	29
6	194.9	79.53	114.97	41
7	194.77	42.02	152.75	22
8	187.47	26.93	160.54	14
9	187.75	101.76	85.99	54
10	194.96	42.56	152.4	22
11	195.11	38.46	156.65	20
12	187.76	48.02	139.74	26
13	194.99	33.16	161.83	17
14	187.34	56.9	130.44	30
15	194.96	4.83	190.13	2
16	187.58	103.31	84.27	55
18	187.69	52.77	134.92	28
19	195.02	63.58	131.44	33
20	187.43	79.47	107.96	42
21	187.67	81.04	106.63	43
22	187.88	48.05	139.83	26
24	193.1	67.7	125.4	35
25	195.88	23.79	172.09	12
26	196.59	29.15	167.44	15
27	187.55	64.82	122.73	35
28	187.35	39.97	147.38	21
29	195.99	33.37	162.62	17
30	187.63	29.5	158.13	16
31	195.41	34.09	161.32	17
32	187.71	29.78	157.93	16
Average	191.11	50.89	140.21	Group: 27 Control: 23 Test: 30

APPENDICES

APPENDIX A

Study ID: HUM00055445 IRB: IRBMED Date Approved: 11/27/2012 Expiration Date: 11/26/2013

UNIVERSITY OF MICHIGAN SCHOOL OF DENTISTRY

Volunteers needed for a gingivitis research study. This study involves collection of oral fluid and plaque samples for measuring markers of gingivitis.

Eligible participants will receive free dental cleanings and compensation.

Major inclusion criteria:

- Age 18-40
- Must have at least 20 permanent teeth
- Non-smoker
- Good general oral health

For more information, please contact:

Michigan Center for Oral Health Research

Phone: (734) 998-6721

E-mail: mcohrclinicalresearch@umich.edu

APPENDIX B

Study ID: HUM00055445 IRB: IRBMED Date Approved: 7/2/2013 Expiration Date: 11/26/2013

UNIVERSITY OF MICHIGAN

CONSENT TO BE PART OF A RESEARCH STUDY

INFORMATION ABOUT THIS FORM

You may be eligible to take part in a research study. This form gives you important information about the study. It describes the purpose of the study, and the risks and possible benefits of participating in the study.

Please take time to review this information carefully. After you have finished, you should talk to the researchers about the study and ask them any questions you have. You may also wish to talk to others (for example, your friends, family, or other doctors) about your participation in this study. If you decide to take part in the study, you will be asked to sign this form. Before you sign this form, be sure you understand what the study is about, including the risks and possible benefits to you.

1. GENERAL INFORMATION ABOUT THIS STUDY AND THE RESEARCHERS

1.1 Study title:

Study of the Anti-Inflammatory Effects of Colgate Total® During an Experimental Gingivitis Model

1.2 Company or agency sponsoring the study:

Colgate-Palmolive Company

1.3 Names, degrees, and affiliations of the researchers conducting the study:

Principal Investigator: Janet Kinney, RDH, MS, MS

Co-Investigator: Diana Kott, RDH

Co-Investigator: Brooke Pancer, DDS

Co-Investigator: William V. Giannobile, DDS, DMSc

Research Staff: Sara Wesley, RDH, BSDH

Study Coordinator: Mary Gilson Layher, RDH, BSDH, CCRP

Study Coordinator: Janet Riggs

2. PURPOSE OF THIS STUDY

2.1 Study purpose:

The overall objective of this proposal is to examine the anti-inflammatory effects of Colgate Total® during an experimental gingivitis model.

3. INFORMATION ABOUT STUDY PARTICIPANTS (SUBJECTS)

Taking part in this study is completely **voluntary**. You do not have to participate if you don't want to. You may also leave the study at any time. If you leave the study before it is finished, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled.

3.1 Who can take part in this study?

If you decide to participate in this study, you will be expected to be present for 5 to 6 appointments. The first 2 visits are needed to see if you qualify for the study.

You must meet the following criteria to be considered for the study: between the ages of 18 to 40; have at least 20 permanent teeth; be a non-smoker. At your first visit you must have a significant amount of gum inflammation and at your second visit you need to have very little gum inflammation.

If you have any of the following you will be excluded: current smoker, quit smoking less than one year ago, or a pack-year history of more than or equal to 10 (pack-years will be calculated by multiplying the number of years smoked by the average number of cigarette-packs smoked per day), antibiotic therapy within 3 months of baseline or the need for antibiotics for infective endocarditis prophylaxis or full joint replacement, chronic medications known to affect the periodontal status (calcium antagonists, anticonvulsives, immunosuppressives, anti-inflammatory medications…), pregnancy or lactating mothers. Women using oral contraceptives are eligible, but those who are new oral contraceptives users within 3 months of baseline or are planning on starting oral contraceptives during the study will be excluded. In particular, subjects using the Depo-Provera contraceptive injection will be excluded due to emerging evidence of increased gingivitis and loss of bone density. A previous reaction to or oral allergy to any ingredient in the study toothpaste; use of any homecare pro ducts to control dental plaque formation within 30 days of your baseline visit; current orthodontic treatment or history of alcoholism or drug abuse, untreated cavities or defective restorations which could worsen during a period of oral hygiene abstinence; and diseases of the immune system or any medical condition that may influence the outcome (diabetes, neurologic or psychiatric disorders, systemic infections…)

3.2 How many people (subjects) are expected to take part in this study?

A total of 30 participants will be enrolled in the study.

4. INFORMATION ABOUT STUDY PARTICIPATION

4.1 What will happen to me in this study?

Visit One

Day -14 (Screening Visit): time will be given for you to read the informed consent and ask any questions related to the study. If you sign the informed consent, we will review your medical and dental history and note any medications you are taking. You will receive a dental examination and the areas around your teeth and gums will be measured using a small dental instrument called a probe. These measurements must fall into a certain range in order for you to qualify. If you qualify, you will be asked to provide a urine sample. A urine analysis will determine your non-smoking status by measuring your body's levels of a substance called cotinine. If your urine analysis results are positive, you will not qualify for the study. An impression of your lower teeth will be taken so that a plastic shield can be created to cover the lower teeth on one side of your mouth. You will then receive a dental cleaning, polishing and oral hygiene instructions.

Visit Two

Day 0 (Baseline Visit): fourteen days after your screening visit subjects will return for their second study visit. We will review your medical history, ask if there have been any changes to any medications you take, and ask if you have had any adverse events since your last study visit. We will then assess the health of your gums to see if they are healthy enough for you to participate in the study. If they are, you will be enrolled in the study. If your gums are not healthy enough, we will review oral hygiene techniques with you and give you an opportunity to return in 2 weeks for a second evaluation. If you return in 2 weeks and your gums are still not healthy enough for you to participate in the study, then you will be excluded from participating in this trial.

If you qualify, you will be randomly assigned into one of two groups of the study (the control group will use a standard of care toothpaste and the test group will use toothpaste with an anti-bacterial agent called Triclosan (Colgate Total®). Neither you nor the examiners will know which group you are in. You will also be randomly assigned to have either right or left side plastic shield made of your lower teeth.

Six intra-oral photos will be taken of your teeth. We will collect saliva and bacterial plaque samples from 2 teeth and take clinical measures of the amount of plaque you have on your teeth and how inflamed your gums are on the sides of your mouth that you are wearing the shields. We will collect a small amount of gum fluid called gingival crevicular fluid from 2 teeth. This is done by placing a tiny paper strip between your tooth and gum tissue for 30 seconds.

You will be instructed to brush your teeth for two minutes twice a day using the assigned study toothpaste. Before brushing, you need to place 2 ml/2.6 g of the assigned toothpaste into the plastic shields and carefully place them in position. The shields will stay in position while you brush the rest of your teeth. After brushing, you need to carefully remove, rinse and store the shields until the next tooth brushing session.

We will give you a toothbrush to use for the duration of the study and instruct you not to use any home oral health care products other than those distributed as part of the study protocol.

Call to Participant

At approximately Day 7 one of the research team members will be contacting by way of a phone call or e-mail to ask how you are doing following the study procedures and to inquire if you are having any problems.

Visit Three

Day 14 Study Visit: you will return after having refrained from tooth brushing your teeth for 10-12 hours before the appointment. We will review your medical history, ask if there have been any changes to any medications you take, and ask if you have had any adverse events since your last study visit. We will take photos of your teeth, collect saliva, bacterial plaque from 2 teeth , collect gingival crevicular fluid from 2 teeth , and assess the amount of plaque and gum inflammation you have on the teeth in the area of the plastic shield. After these measurements have been made, you will put the plastic shield into position with the toothpaste inside of the shield and brush your teeth for 2 minutes. Gingival crevicular fluid samples will be collected again from the same 2 teeth at approximately 1, 2, 4, and 6 hours post-brushing.

We will remind you to continue at-home study tooth brushing procedures until next study visit.

You will receive $100 incentive payment.

Visit Four

Day 21 Study Visit: you will return after having refrained from tooth brushing your teeth for 10-12 hours before the appointment. We will review your medical history, ask if there have been any changes to any medications you take, and ask if you have had any adverse events since your last study visit. We will take photos of your teeth, collect saliva, bacterial plaque from 2 teeth , collect gingival crevicular fluid from 2 teeth, and assess the amount of plaque and gum inflammation you have on the teeth in the area of the plastic shield. After these measurements have been made, you will put the plastic shield into position with the toothpaste inside of the

shield and brush your teeth for 2 minutes. Gingival crevicular fluid samples will be collected again from the same 2 teeth at approximately 1, 2, 4, and 6 hours post-brushing.

You will receive a full-mouth dental cleaning.

We will collect your plastic shields and any unused toothpaste. A quantity of more than 60% will be considered lack of compliance with the study protocol and grounds for dismissal from the study.

You will receive $100 incentive payment.

Visit Five

Day 35 Study Visit: you will return after having refrained from tooth brushing your teeth for 10-12 hours before the appointment. We will review your medical history, ask if there have been any changes to any medications you take, and ask if you have had any adverse events since your last study visit. We will take photos of your teeth, collect saliva, bacterial plaque from 2 teeth, collect gingival crevicular fluid from 2 teeth , and assess the amount of plaque and gum inflammation you have on the teeth in the area of the plastic shield.

You will receive $300 incentive payment.

4.2 How much of my time will be needed to take part in this study?

Your participation in this study will last for approximately 7 ½ weeks. There are 5 to 6 study visits involved in this project. Your visit today should take about 2 hours. The second study visit will take approximately 1 hour. Study visits three and four will take about 7 hours. At these two visits you will have flexibility to leave the research facility as long as you return 1,2,4,6 hours post-brushing. Your final study visit should last about 1 ½ hours.

4.3 When will my participation in the study be over?

Your participation in the study will end after your final study visit.

5. INFORMATION ABOUT RISKS AND BENEFITS

5.1 What risks will I face by taking part in the study? What will the researchers do to protect me against these risks?

There are minimal risks or discomforts associated with collection of saliva and dental plaque samples.

The known or expected risks are:

Risks associated with dental cleanings are small. Possible risks include minimal discomfort and bleeding associated with scaling of the teeth.

Overall, these types of studies have been proven to be safe with relatively minimal adverse side effects. Adverse effects include bleeding of the gums, plaque accumulation and bad breath. Studies have shown that these effects are completely reversible when oral hygiene is resumed after 21 days. Subjects are expected to reverse back to a state of oral health within 7 days when the oral hygiene is resumed.

In cases of existing dental conditions (e.g. cavities, untreated gum diseases), the disease can worsen if the oral hygiene is discontinued. For this reason, such subjects will be excluded from the study.

The researchers will try to limit these risks by: closely monitoring you for the duration of the study and providing a full mouth dental cleaning at the beginning of the study and at Day 21.

As with any research study, there may be additional risks that are unknown or unexpected.

5.2 What happens if I get hurt, become sick, or have other problems as a result of this research?

The researchers have taken steps to minimize the risks of this study. Even so, you may still have problems or side effects, even when the researchers are careful to avoid them. Please tell the researchers listed in Section 10 about any injuries, side effects, or other problems that you have during this study. You should also tell your regular doctors**.**

5.3 If I take part in this study, can I also participate in other studies?

Being in more than one research study at the same time, or even at different times, may increase the risks to you. It may also affect the results of the studies. You should not take part in more than one study without approval from the researchers involved in each study.

5.4 How could I benefit if I take part in this study? How could others benefit?

You may not receive any personal benefits from being in this study. Future patients may benefit as a result of this research due to dentists being able to more easily assess patients' oral health.

5.5 Will the researchers tell me if they learn of new information that could change my willingness to stay in this study?

Yes, the researchers will tell you if they learn of important new information that may change your willingness to stay in this study. If new information is provided to you after you have joined the study, it is possible that you may be asked to sign a new consent form that includes the new information.

6. OTHER OPTIONS

6.1 If I decide not to take part in this study, what other options do I have?

If you decide not to participate in this study you have the option of returning to your private dental practice for ongoing oral health care needs.

7. ENDING THE STUDY

7.1 If I want to stop participating in the study, what should I do?

You are free to leave the study at any time. If you leave the study before it is finished, there will be no penalty to you. You will not lose any benefits to which you may otherwise be entitled. If you choose to tell the researchers why you are leaving the study, your reasons for leaving may be kept as part of the study record. If you decide to leave the study before it is finished, please tell one of the persons listed in Section 10 “Contact Information” (below).

7.2 Could there be any harm to me if I decide to leave the study before it is finished?

No, there will be no harm to you if you decide to leave the study before it is finished, but we would like to schedule a final visit to ensure your teeth and gums are healthy before you leave.

7.3 Could the researchers take me out of the study even if I want to continue to participate?

Yes. There are many reasons why the researchers may need to end your participation in the study. Some examples are:

- ✓ The researcher believes that it is not in your best interest to stay in the study.
- ✓ You become ineligible to participate.
- ✓ Your condition changes and you need treatment that is not allowed while you are taking part in the study.
- ✓ You do not follow instructions from the researchers.
- ✓ The study is suspended or canceled.

✓ 8. FINANCIAL INFORMATION

8.1 Who will pay for the costs of the study? Will I or my health plan be billed for any costs of the study?

The study will pay for research-related items or services that are provided only because you are in the study. If you are not sure what these are, see Section 4.1 above or ask the researchers for a list. If you get a bill you think is wrong, call the researchers' number listed in section 10.1.

You or your health plan will pay for all the things you would have paid for even if you were not in the study, like:

- Health care given during the study as part of your regular care
- Items or services needed to give you study drugs or devices
- Monitoring for side effects or other problems
- Deductibles or co-pays for these items or services.

If you do not have a health plan, or if you think your health plan may not cover these costs during the study, please talk to the researchers listed in Section 10 below or call your health plan's medical reviewer.

By signing this form, you do not give up your right to seek payment if you are harmed as a result of being in this study.

8.2 Will I be paid or given anything for taking part in this study?

Yes, you will be paid $500 for your participation in the study. Eligible subjects will receive $100 at Day 14, $100 at Day 21, $300 at Day 35.

8.3 Who could profit or financially benefit from the study results?

The company whose product is being studied: Colgate-Palmolive Company

9. CONFIDENTIALITY OF SUBJECT RECORDS AND AUTHORIZATION TO RELEASE YOUR PROTECTED HEALTH INFORMATION

The information below describes how your privacy and the confidentiality of your research records will be protected in this study.

9.1 How will the researchers protect my privacy?

If you qualify for the study you will be given a study number. We will use your study number along with your initials on any samples we take and on all of our data collection systems.

9.2 What information about me could be seen by the researchers or by other people? Why? Who might see it?

Signing this form gives the researchers your permission to obtain, use, and share information about you for this study, and is required in order for you to take part in the study. Information about you may be obtained from any hospital, doctor, and other health care provider involved in your care, including:

- Hospital/doctor's office records, including test results (X-rays, blood tests, urine tests, etc.)

- All records relating to your condition, the treatment you have received, and your response to the treatment
- Billing information

There are many reasons why information about you may be used or seen by the researchers or others during or after this study. Examples include:

- The researchers may need the information to make sure you can take part in the study.
- The researchers may need the information to check your test results or look for side effects.

- University, Food and Drug Administration (FDA), and/or other government officials may need the information to make sure that the study is done in a safe and proper manner.

- Study sponsors or funders, or safety monitors or committees, may need the information to:
    - Make sure the study is done safely and properly
    - Learn more about side effects
    - Analyze the results of the study
- The researchers may need to use the information to create a databank of information about your condition or its treatment.
- Information about your study participation may be included in your regular UMHS medical record.
- If you receive any payments for taking part in this study, the University of Michigan accounting department may need your name, address, social security number, payment amount, and related information for tax reporting purposes.
- Federal or State law may require the study team to give information to government agencies. For example, to prevent harm to you or others, or for public health reasons.

The results of this study could be published in an article, but would not include any information that would let others know who you are.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

9.3 What happens to information about me after the study is over or if I cancel my permission?

As a rule, the researchers will not continue to use or disclose information about you, but will keep it secure until it is destroyed. Sometimes, it may be necessary for information about you to continue to be used or disclosed, even after you have canceled your permission or the study is over.

Examples of reasons for this include:

- To avoid losing study results that have already included your information
- To provide limited information for research, education, or other activities (This information would not include your name, social security number, or anything else that could let others know who you are.)
- To help University and government officials make sure that the study was conducted properly

As long as your information is kept within the University of Michigan Health System, it is protected by the Health System's privacy policies. For more information about these policies, ask for a copy of the University of Michigan "Notice of Privacy Practices". This information is also available on the web at http://www.med.umich.edu/hipaa/npp.htm. Note that once your information has been shared with others as described under Question 9.2, it may no longer be protected by the privacy regulations of the federal Health Insurance Portability and Accountability Act of 1996 (HIPAA).

9.4 When does my permission expire?

Your permission expires at the end of the study, unless you cancel it sooner. You may cancel your permission at any time by writing to the researchers listed in Section 10 "Contact Information" (below).

10. CONTACT INFORMATION

10.1 Who can I contact about this study?

Please contact the researchers listed below to:

- Obtain more information about the study
- Ask a question about the study procedures or treatments
- Talk about study-related costs to you or your health plan
- Report an illness, injury, or other problem (you may also need to tell your regular doctors)
- Leave the study before it is finished
- Express a concern about the study

Principal Investigator: Janet Kinney, RDH, MS, MS
Mailing Address: 24 Frank Lloyd Wright Dr, Lobby M, Box 442, Ann Arbor, MI 48106

Telephone: (734) 998-1468

Research Core Staff

Michigan Center for Oral Health Research (MCOHR)
Mailing Address: 24 Frank Lloyd Wright Drive, Lobby M, Box 442, Ann Arbor, MI 48106

Telephone: (734) 998-6721

You may also express a concern about a study by contacting the Institutional Review Board listed below.

University of Michigan Medical School Institutional Review Board (IRBMED)
2800 Plymouth Road
Building 200, Room 2086
Ann Arbor, MI 48109-2800

Telephone: 734-763-4768 (For International Studies: US Country Code: 001)
Fax: 734-763-1234
e-mail: irbmed@umich.edu

If you are concerned about a possible violation of your privacy or concerned about a study you may contact the University of Michigan Health System Compliance Help Line at 1-866-990-0111.

When you call or write about a concern, please provide as much information as possible, including the name of the researcher, the IRBMED number (at the top of this form), and details about the problem. This will help University officials to look into your concern. When reporting a concern, you do not have to give your name unless you want to.

11. RECORD OF INFORMATION PROVIDED

11.1 What documents will be given to me?

Your signature in the next section means that you have received copies of all of the following documents:

- This "Consent to be Part of a Research Study" document. (*Note: In addition to the copy you receive, copies of this document will be stored in a separate confidential research file and may be entered into your regular University of Michigan medical record.)*
- Other (specify):_____

12. SIGNATURES

Photographs

I understand that photographs will be taken as part of the study procedures on Days 0, 14, 21, and 35. Reasonable efforts will be made to conceal any features that would disclose or reveal my identity. I understand that these photographs may be published for educational and scientific purposes. I have indicated below if I am willing to allow my photographs to be published for educational and scientific purposes.

☐ YES, **I DO consent** to photographs of my oral and facial structures being published for educational and scientific purposes.

☐ NO, **I DO NOT consent** to photographs of my oral and facial structures being published for educational and scientific purposes.

Research Subject:
I understand the information printed on this form. I have discussed this study, its risks and potential benefits, and my other choices with _____. My questions so far have been answered. I understand that if I have more questions or concerns about the study or my participation as a research subject, I may contact one of the people listed in Section 10 (above). I understand that I will receive a copy of this form at the time I sign it and later upon request. I understand that if my ability to consent for myself changes, either I or my legal representative may be asked to re-consent prior to my continued participation in this study.

Name (print legal name): _____

Signature of Subject: _____

Date of signature: _____

Patient ID: _____ *Date of Birth (mm/dd/yyyy):* _____

Principal Investigator (or Designee):
I have given this research subject (or his/her legally authorized representative, if applicable) information about this study that I believe is accurate and complete. The subject has indicated that he or she understands the nature of the study and the risks and benefits of participating.

Name: _____

Title: _____

Signature: _____

Date of Signature: _____

APPENDIX C

Study ID: HUM00055445 IRB: IRBMED Date Approved: 11/27/2012 Expiration Date: 11/26/2013

Experimental Gingivitis Phone Script

Hello, my name is_____. I am calling from Michigan Center for Oral Health Research (MCOHR) regarding the dental study that you are interested in. Do you have a few minutes to discuss the study?

This research study is being done to measure the levels of certain proteins and bacteria related to gingivitis (inflammation of the gums). Previous studies have shown that these substances are found at higher levels in areas of inflammation. We are recruiting 30 patients for this study. This study will include 5 to 6 visits and last approximately 2 months. Most of the study visits will last between 1-2 hours, but two of the study visits will last about 7 hours.

If you are eligible, we will take an impression of your lower teeth for the preparation of a plastic shield you will need to wear twice a day when you brush your teeth. You must wear the shield every time you brush your teeth. You will also have a dental cleaning.

You will return to the clinic on days 14, 21, and 35. We will take photos of your mouth and measure the levels of gum inflammation. Saliva, bacterial samples, and gum tissue fluid samples will be collected. You will be asked to place a small amount of toothpaste into the plastic shield and then brush the rest of your teeth for 2 minutes. On days 14 and 21 we will repeat taking gum tissue fluid samples 12, 4, and 6 hours after you brush.These study visits will last approximately 7 hours. You will not need to stay in the dentalclinic for the entire time, but will need to return to the dental clinic at the previously
mentioned times so that the oral fluids can be collected at the appropriate time intervals. At Day 21, you will receive a thorough dental cleaning and can resume your normal oral health care routine.

You will return on day 35 for your last study visit. We will collect all of the same samples, measure the amount of inflammation in your gum tissues and check to make sure that your gums have returned to their pre-study levels of health.

If enrolled, you will receive $500 for a total of 5 to 6 visits. You will receive $100 at your Day 14 Study Visit, $100 at your Day 21 Study Visit, and $300 at your Day 35 Study Visit.

I would like to go over some preliminary inclusion criteria with you. I will be asking you questions about your personal health and child bearing potential. Please only answer questions that you want to answer. Answering is optional, and you can tell me to stop asking these questions at any time. The purpose of these questions is to pre-screen subjects to see if they are likely to qualify for the study. We will make notes in our subject database as to whether or not you appear to qualify for the study. This information will be used to search for potential subjects for future studies. Would you like me to continue?

- Are you between 18 and 40 years old? (must be 40 for the duration of the patient's involvement in the study)
- Do you have at least 20 permanent teeth?
- Do you have good general oral health?
- Are you a non-smoker?
- If you quit smoking less than a year ago, you are excluded from the study
- Have you smoked in the past? If so, how much and for how long? if a previous smoker – must have quit smoking over 1 year and have a pack-year history equal or less than 10 (pack-years is calculated by multiplying the number of years smoked by the average number of packs per day)
    - We will be verifying your non-smoking status by urinalysis should you decide to screen for the study.
    - Reminder: Nicorette gum and marijuana use in the last year will also exclude patient; In case patient does provide this information.
- Have you been on antibiotic therapy within 3 months of baseline, need antibiotics for infective endocarditis prophylaxis or total joint replacement?
- Are you taking medications known to affect periodontal status (calcium antagonists, anticonvulsives, immunosuppressives, or anti-inflammatory medications)?
- Are you pregnant or lactating?
- Are you currently receiving orthodontic or periodontal treatment?
- Do you have history of alcohol or drug abuse?
- Do you have untreated cavities?
- Do you have any systemic infections or immune system disorders (diabetes, neurologic, psychiatric disorders....)?
- If using oral or other hormonal contraceptives (ex: the pill, patch, NuvaRing), must be on for more than 3 months

- Are you using Depo-Provera injections?
- If you are a pre-menopausal woman you or your male partner must be surgically sterile or you must be using reliable birth control (ie prescription oral contraceptives, contraceptive injections, intrauterine device, double-barrier method, contraceptive patch) now and throughout the study or abstain from sex throughout the study
- Do you have an active oral infection, including periodontitis?
- Do you have a known reaction to or oral allergy to any ingredient in the study toothpaste?
- Have you used an antiseptic homecare oral product to control dental plaque formation? (must not be within 30 days prior to the baseline visit)

If patient does not qualify:

Would you like to hear about the other studies at our center?

If yes, continue with description of other studies.

If no, continue to last 2 paragraphs of this script.

If patient does qualify:

If you are interested, I would like to schedule a screening appointment for you.

Are you a patient at the University of Michigan Dental School? If so, when were you last seen there? If you are not a U of M dental school patient, we will need to enter your information into our scheduling system now. **Open axiUm and enter patient information**

Do you know where Domino's Farms is located? We are in Lobby M on the second floor. Come through the glass doors and the receptionist will greet you there. Do you need a map?

Since we schedule an individual appointment for each screening patient, please let us know within 24 hours if you are unable to keep your appointment so we can schedule another person for that time. Our number is (734) 998-6721.

Please plan on the screening appointment taking up to 2 hours. Please arrive 15 minutes early to your visit to fill out paperwork. If you are more than 20 minutes late to the scheduled visit, you will not be seen due to time constraints.

As a reminder, the information we discussed today will be stored in our research subject database. It will remain there so we can easily find subjects to screen for future studies. Your personal contact information is also kept in the dental school scheduling database. Do you have any questions regarding how your personal information is handled?

Thank you very much for your interest in MCOHR studies.

End if patient did not qualify for any studies.

Continue if patient qualified for a study.

Thank you, and we will see you on _____

APPENDIX D

Day 7 Examiner:

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (if "Yes" complete AE form)	**YES**	**NO**

Dear _____,

This is _____ from the Michigan Center for Oral Health Research (MCOHR). I am calling/writing to see how things are going for you with the experimental gingivitis study you are enrolled in. Do you have any questions about wearing the plastic shield? Have you been following the instructions for using the plastic shields when brushing your teeth? Do you have any concerns about your participation in the study at this time? Have you been brushing twice a day?

You are scheduled for your Day 14 study visit on _____. Please remember not to brush your teeth for 10 to 12 hours before your appointment. Also, please remember that at this visit will last approximately 7 hours. We will be sampling oral fluids 1, 2, 4, and 6 hours after you brush your teeth, so if you decide to leave MCOHR between sampling times, you return on time so that we are able to collect the oral fluids according to the study protocol.

Once again, thank you for participating in the study. You can call us at 734-998-6712 if you have any questions or concerns.

We look forward to seeing you again in a week.

***Reminder* - Ask subject to bring in stent and toothpaste at next appointment.**

APPENDIX E

Randomization List

Patient ID	Stent	Day 0		Day 14		Day 21		Day 35	
1	Right	31D	30M	31M	28M	30D	29M	28D	29D
2	Left	19D	18D	20D	18M	21M	20M	21D	19M
3	Left	20M	18D	19M	18M	21D	21M	20D	19D
4	Right	29D	30D	28D	30M	31D	31M	28M	29M
5	Left	21D	18M	20D	21M	18D	20M	19D	19M
6	Left	21M	20D	20M	19D	19M	18M	18D	21D
7	Right	30D	29M	29D	31M	31D	30M	28M	28D
8	Right	31D	29D	30M	28M	31M	28D	30D	29M
9	Right	31D	30D	28D	30M	29M	28M	31M	29D
10	Left	18D	18M	19M	21M	20M	20D	19D	21D
11	Right	29M	29D	28M	31M	30D	30M	28D	31D
12	Left	20D	21M	18D	20M	19D	19M	21D	18M
13	Left	18D	21D	20D	18M	20M	19D	21M	19M
14	Right	30D	29M	30M	31D	31M	28D	28M	29D
15	Right	30D	28D	28M	30M	31D	31M	29M	29D
16	Left	19D	20D	21D	21M	19M	20M	18D	18M
17	Left	21D	21M	19M	18D	18M	20M	20D	19D
18	Right	30D	29D	31D	28M	31M	28D	29M	30M
19	Right	31D	29D	30M	29M	31M	28M	30D	28D
20	Left	18D	20M	21D	19M	21M	18M	19D	20D
21	Left	18M	21D	20M	19M	18D	21M	20D	19D
22	Right	28M	30D	29D	31D	29M	31M	30M	28D
23	Right	28M	30M	31D	29M	31M	29D	28D	30D
24	Left	20D	20M	21D	19M	18M	21M	19D	18D
25	Right	28D	30D	28M	31M	30M	31D	29D	29M
26	Left	18D	20M	21D	21M	18M	20D	19D	19M
27	Left	18D	20D	20M	19D	21M	21D	19M	18M
28	Right	28M	31M	29M	30D	29D	31D	30M	28D
29	Left	18M	19M	19D	21M	21D	20D	18D	20M
30	Right	31M	28M	31D	29D	30M	29M	28D	30D
31	Right	29D	28D	30D	30M	28M	31M	31D	29M
32	Left	19D	19M	21D	18M	18D	21M	20M	20D
33	Right	31M	29M	30D	31D	29D	28M	28D	30M
34	Left	21M	19M	20D	18M	19D	20M	21D	18D
35	Right	28D	30M	31M	29M	29D	28M	30D	31D
36	Left	21D	19M	20D	19D	21M	20M	18M	18D
37	Left	21D	21M	18D	20M	20D	19D	19M	18M
38	Right	31M	28D	30M	30D	31D	29D	29M	28M
39	Left	20M	20D	19M	18M	21M	19D	21D	18D
40	Right	29M	28M	29D	31M	30D	30M	31D	28D

APPENDIX F

Dear Previous MCHOR Research Patient:

We are writing to invite you to participate in a research study for inflammation of the gums (gingivitis). The purpose of this research study is to examine the anti-inflammatory effects of Colgate Total during an experimental gingivitis model. The study will require 5 to 6 visits and will last for approximately 2 months. Over the course of the study, you will be compensated for your participation.

You May be eligible if:

- You are between the ages of 18 and 40
- You have at least 20 teeth
- You do NOT have an allergy to Colgate Total
- You do NOT need to take antibiotics before dental treatment
- You have NOT taken any antibiotics in the last 3 months
- You have NOT had periodontal treatment in the last 6 months (regular cleanings are acceptable)
- You have NOT used tobacco products in the past year, such as cigarettes, cigars, chew
- You do NOT have any of the following medical conditions: diabetes, HIV infection, AIDS
- You are NOT pregnant, lactating or breastfeeding

If you think you may be interested in our new study, please call us at (734) 998 – 1468 so that we may provide you with additional information and/or carry out a phone screening at your convenience. Thank you for your interest in our clinical research at the Michigan Center for Oral Health Research (MCOHR)!

Kindest Regards,

Janet Riggs
Michigan Center for Oral Health Research (MCOHR)
University of Michigan
24 Frank Lloyd Wright Drive
Lobby M, Box 422
Ann Arbor, MI 48106
janetrig@umich.edu

APPENDIX G

COLGATE
ADVERSE EVENT TRACKING LOG

IRB Protocol #: HUM00055445	**Study Title:** Study of the Anti-Inflammatory Effects of Colgate Total® During an Experimental Gingivitis Model
Investigator Name: Janet Kinney	**Study Coordinator Name: Jan Riggs**

	Subject ID	Start Date of Event	End Date (indicate if ongoing)*	Event **				Date Report sent to IRB	Date Report sent to Sponsor (if applicable)
				Description	Severity	Relationship	Outcome		
1	01	30/Mar/13	06/Apr/13	Aphthous ulcer	Mild	Possible; may be related to taking stent in & out of mouth	Resolved		
2	03	07/Apr/13	26/Apr/13	Tissue Sloughing	Mild	Possible	Resolved		
3	11	05/Aug/13	Unknown	Aphthous ulcer	Mild	Possible	Resolved		
4	16	14/Aug/13	19/Aug/13	Left TMJ soreness	Mild	Unknown	Resolved		
5	17	09/Aug/13	Unknown	Decay #18	Mild	Unrelated	Unknown		
6	21A	04/Sept/13	Ongoing	Tingling in tongue	Mild	Possible	Resolved		
7	21B	25/Aug/13	Unknown	Aphthous Ulcer	Mild	Possible	Resolved		
8	22	Unknown	Ongoing	Toothbrush trauma	Mild	Unrelated	Resolving		
9	30	05/Nov/13	12/Nov/13	Herpetic lesion	Mild	Possibly	Resolved		
10	27	06/Oct/13	21/Oct/13	Aphthous ulcer	Mild	Possibly	Resolved		
11	28	28/Oct/13	04/Nov/13	Herpetic lesion	Mild	Possibly	Resolved		
12	31	26/Nov/13	30/Nov/13	Tissue sloughing	Mild	Possibly	Resolved		

* All events should be resolved or noted as ongoing at the time of subjects discontinuation in the study (i.e. study complete, subject withdrawal, death)

** Report all adverse events in accordance with UMMS IRB Policy : http://med.umich.edu/irbmed/ae_orio/ae_report.htm

Original: Regulatory File **Copy:** As Needed

Revised 01Sep2010

APPENDIX H
Screening (Day -14) Examination Form

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	Date of Screening (MON/DD/YY) / /	Patient Number	Patient Initials

Informed Consent

Was the informed consent form signed? ☐ Yes ☐ No Initials _____

Was the informed consent documented in the treatment record? ☐ Yes ☐ No Initials _____

Was a copy given to the patient? ☐ Yes ☐ No Initials _____

General Inclusion Criteria:	Yes	No
1. Is the subject aged 18 through 40 years?	☐	☐
2. Does the subject willing to follow all study procedures?	☐	☐
3. Does the subject have a minimum of 20 permanent teeth?	☐	☐
4. Does the subject have probing pocket depths ≤ 4mm on all sites?	☐	☐
5. Does the subject have a mean clinical attachment level of ≤ 2mm?	☐	☐
6. Does the subject have a mean score of ≤ 1 in both the plaque and gingival indices?	☐	☐
7. Does the subject have a BOP score ≥ 30 percent?	☐	☐

General Exclusion Criteria: **Yes** **No**

1. Is the subject a current smoker, quit smoking less than one year ago, or have a pack-year history of more than or equal to 10 (pack-years will be calculated by multiplying the number of years smoked by the average number of cigarette packs smoked per day)? ☐ ☐

   Pack-year History Calculation:

   _____ packs of cigarettes smoked per day (times) ___ number of years smoked = _____

   Pack-year

   History

2. Has the subject had antibiotic therapy within 3 months of the baseline visit or need antibiotics for infective endocarditis prophylaxis? ☐ ☐
3. Does the subject take chronic medications known to affect the periodontal status (calcium antagonists, anticonvulsives, immunosuppressives, anti-inflammatory medications….)? ☐ ☐
4. Is the subject pregnant or lactating? ☐ ☐
5. Is the subject currently in active orthodontic or periodontal treatment? ☐ ☐
6. Does the subject have a history of alcoholism or drug abuse? ☐ ☐
7. Does the subject have any untreated carious lesions or defective restorations which could exacerbate during a period of oral hygiene abstinence? ☐ ☐
8. Does the subject have any disease of the immune system or any medical condition that may influence the outcome (diabetes, neurologic, psychiatric disorders, systemic infections…)? ☐ ☐
9. Is the subject a new oral contraceptive user (within 3 months of baseline) or plan to start taking oral contraceptives during the study? ☐ ☐
10. Does the subject use Depo-Provera contraceptive injections? ☐ ☐

General Information

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date of Screening** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

Date of Birth MON/DD/YY
/ /

Gender
M F

Race:	Caucasian	African American	Asian	Hispanic	Native American	Other

Medical/Oral History
Was the medical/oral history recorded? ☐ Yes ☐ No

Oral Soft Tissue Examination
Was oral soft tissue exam performed? ☐ Yes ☐ No

Laboratory
Was a urine analysis taken? ☐ Yes ☐ No

Clinical
Was a prophylaxis preformed? ☐ Yes ☐ No

Was the subject given OHI? ☐ Yes ☐ No

Impression

Was a mandibular impression taken for stent? ☐ Yes ☐ No

_____ _____

Investigator Signature Date

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

CLINICAL (Calculation on mean on page 11)

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
FGM																					
PD																					
CAL																					
BOP																					
BUCCAL	**2**			**3**			**4**			**5**			**6**			**7**			**8**		
FGM																					
PD																					
CAL																					
BOP																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
FGM																					
PD																					
CAL																					
BOP																					
LINGUAL	**15**			**14**			**13**			**12**			**11**			**10**			**9**		
FGM																					
PD																					
CAL																					
BOP																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

CLINICAL (Calculation on mean on page 11)

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
FGM																					
PD																					
CAL																					
BOP																					
BUCCAL	**18**			**19**			**20**			**21**			**22**			**23**			**24**		
FGM																					
PD																					
CAL																					
BOP																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
FGM																					
PD																					
CAL																					
BOP																					
LINGUAL	**31**			**30**			**29**			**28**			**27**			**26**			**25**		
FGM																					
PD																					
CAL																					
BOP																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

PLAQUE INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
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PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean PLAQUE INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria
0	No plaque
1	A film of plaque adhering to free gingival margin and adjacent area of tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

GINGIVAL INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**L**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean GINGIVAL INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria	Bleeding
0	Absence of inflammation	No bleeding on probing
1	Mild inflammation Slight change in color and texture	No bleeding on probing
2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Percentage BLEEDING ON PROBING SCORE for Patient:

Number of BOP Sites ÷ Total Number of Sites x 100 = PERCENTAGE

_____ ÷ _____ x 100 = _____

Score	Criteria
0	No bleeding upon probing
1	Presence of bleeding upon probing

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

CALCULATIONS:

Mean Clinical Attachment

Total ÷ Number of sites = MEAN _____ ÷ _____ = _____

RESULTS:

1) Mean CLINICAL ATTACHMENT = _____

2) Mean PLAQUE INDEX = _____

3) Mean GINGIVAL INDEX = _____

4) Percentage BLEEDING ON PROBING SCORE = _____

APPENDIX I

Baseline (Day 0) Examination Form

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (if "Yes" complete AE form)	**YES**	**NO**

Medical/Oral History **Yes** **No**

Was the medical/oral history updated? ☐ ☐

Oral Soft Tissue Exam

Were photographs taken? ☐ ☐

Was an oral soft tissue exam performed? ☐ ☐

Bleeding on Probing

Did the subject have a BOP score ≤ 10 percent (If answered "No", subject needs to return in two weeks for a second assessment visit.)? ☐ ☐

Qualification **Yes** **No**

Does the subject qualify for the study? ☐ ☐

Study Procedures

Whole Saliva **Yes** **No**

Was whole saliva collected? ☐ ☐

_____ ml collected in _____ minutes (max. 15)

Plaque Index

Was the Plaque Index (PI) completed? ☐ ☐

The mean PI was _____

Gingival Index

Was the Gingival Index (GI) completed? ☐ ☐

The mean GI was _____

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

	Yes	No
Gingival Crevicular Fluid Was a gingival crevicular fluid sample collected pre-brushing?	☐	☐
Which teeth were sampled? ____ ____ ____ ____		
Plaque Biofilm Was sub-gingival plaque and GCF samples collected?	☐	☐
Which teeth were sampled? ____ ____ ____ ____		
Oral Hygiene		
Was the subject instructed to brush their teeth in the non-stent area for two minutes twice a day using the assigned study toothpaste?	☐	☐
Was the subject instructed to place 2ml/2.6g of assigned toothpaste into the stent before brushing?	☐	☐
Was the subject instructed to keep the stent in position while the rest of the mouth is cleaned?	☐	☐
Was the subject instructed to carefully remove, rinse and store the stent after brushing?	☐	☐
Was the subject instructed not to use any home oral health care products other than those distributed as part of the study?	☐	☐

_____ _____

Investigator Signature **Date**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

PLAQUE INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
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PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean PLAQUE INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria
0	No plaque
1	A film of plaque adhering to free gingival margin and adjacent area of tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

GINGIVAL INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean GINGIVAL INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria	Bleeding
0	Absence of inflammation	No bleeding on probing
1	Mild inflammation Slight change in color and texture	No bleeding on probing
2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

BLEEDING ON PROBING SCORE

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
BOP																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
BOP																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
BOP																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
BOP																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
BOP																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
BOP																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
BOP																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
BOP																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Percentage BLEEDING ON PROBING SCORE for Patient:

Number of BOP Sites ÷ Total Number of Sites x 100 = PERCENTAGE

_____ ÷ _____ x 100 = _____

Score	Criteria
0	No bleeding upon probing
1	Presence of bleeding upon probing

APPENDIX J

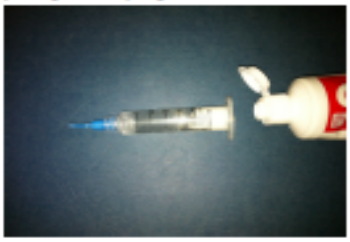
Power Point Instructions For At-Home Use of Dentifrice and Stent During Experimental Gingivitis Phase

4/10/14

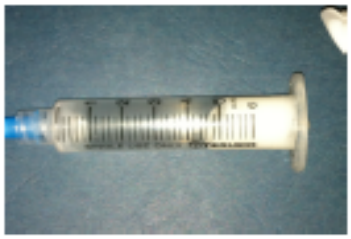
Experimental Gingivitis:
Visual Illustrations

Participant Homecare Instructions

Remove the plunger from syringe tube by pulling the handle back. Squeeze assigned toothpaste into the large opening of the syringe tube.



Fill syringe until the toothpaste reaches the 5cc mark.



Place the plunger back into the syringe tube and push toothpaste forward until the black stopper reaches the 2cc mark.

If there is excess toothpaste in the syringe, squeeze excess back into the assigned toothpaste tube.

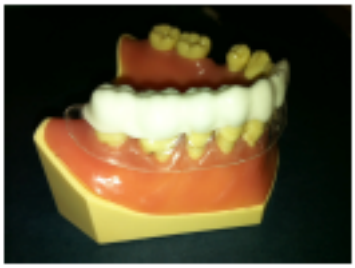
If there is excess toothpaste in the syringe, squeeze excess back into the assigned toothpaste tube.

Fill the stent by pushing the plunger forward. Fill the stent as evenly as possible on the bottom, then move on to fill the impression of the teeth. Fill the stent such that all the toothpaste is emptied from the syringe.

Fill the stent such that all the toothpaste is emptied from the syringe.

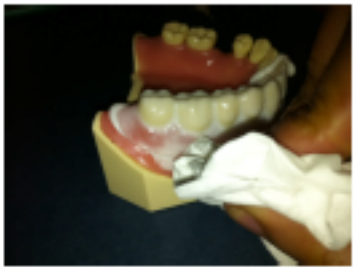
Squeeze a ribbon of toothpaste on your toothbrush.

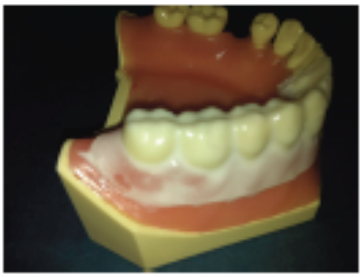
Place the toothpaste-filled stent into your mouth.



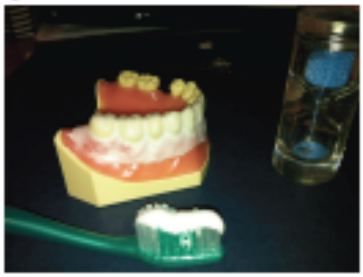
Push the stent completely down until it fits comfortably over the teeth.

Wipe any excess toothpaste off your gums.





Turn the 2-minute timer over to begin. Brush the non-stent areas of your mouth for the 2 full minutes. Avoid brushing the stent area.



After you have brushed the non-stent areas for 2 minutes, remove the stent from your mouth. Rinse your mouth thoroughly to remove any extra toothpaste.

Rinse your stent using warm (not hot) water.

Place your stent back in the stent holder

Complete the brushing procedures twice a day – once in the morning and once at night.

Please call us if you have any questions.

Study Coordinator: Janet Riggs, (714) 898-1468.

APPENDIX K

Take Home Instructions Sheet for At-Home Use of Dentifrice and Stent During Experimental Gingivitis Phase

Experimental Ginvigitis Study

1. Remove plunger from syringe tube
2. Squeeze assigned toothpaste into syringe tube such that the tube evenly fills up from the end to the 5cc mark
3. Place plunger back in end of syringe tube and push forward until black stopper reaches the toothpaste
4. Verify that the toothpaste is filled to the 2cc mark (the black stopper should end at that line too)
    a. If there is excess toothpaste in the syringe (i.e. the toothpaste and black stopper end closer to the plunger end than the 2cc line)- squeeze out the excess toothpaste into the assigned toothpaste tube by pushing the plunger forward until it stops at the 2cc mark
    b. If there is insufficient toothpaste in the syringe (i.e. the toothpaste and black stopper end closer to the nozzle tip than the 2cc line)- remove the plunger from the syringe, squeeze the desired amount of toothpaste into the syringe such that when the plunger is replaced and pushed forward, the black stopper stops at the desired 2cc mark
5. Hold the stent with you non-dominant hand with the open end facing you
6. With your dominant hand, push the plunger forward to as to squeeze the assigned toothpaste into the stent first starting at the very base, filling it as evenly as possible on the bottom, then moving on to fill the side walls of the stent. Fill the stent such that all the toothpaste is expelled into the stent
7. Before placing the stent in the assigned area of the mouth, place it aside on a flat counter top with the open end facing the ceiling

8. With your assigned toothbrush, slightly wet it to soften the bristles. Place a ribbon of the assigned toothpaste along the length of the bristles in an even manner
9. Set the toothbrush aside on a flat countertop
10. Have your timer ready
11. Place the stent in the assigned area of your mouth and push down all the way until it is seated comfortably
12. Carefully wipe off any excess toothpaste that expelled out of the stent onto your gums with a piece of tissue
13. Turn the time over so as to start the 2 minute hygiene session
14. Brush the remaining 3 quadrants of your mouth (not the area of the mouth with the stent) using a circular scrub technique as instructed earlier
15. When 2 minutes has elapsed as indicated by the timer, remove the toothbrush and stent from your mouth
16. Gently rinse your mouth with water to remove any remaining toothpaste
17. Rinse the toothbrush, stent, and disassembled syringe with warm water. Do not use hot water for this process as it can melt the plastics. The toothbrush can also be used to remove any remaining toothpaste from the stent
18. Place the clean stent in the case provided. Place the assigned toothpaste, stent, and toothbrush in the bag provided for safekeeping
19. *Do not use dental floss, any interproximal cleaning devices (such as a Waterpik®, ProxaBrush®, toothpick etc…), or mouthwash anywhere in your mouth throughout the duration of the study*
20. *No home oral health care products other than those distributed as part of the study protocol may be used (only the assigned toothpaste and toothbrush may be used)*

APPENDIX L

Day 14 Examination Form

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (if "Yes" complete AE form)	**YES**	**NO**

	Yes	**No**
Tooth Brushing Has the subject refrained from brushing their teeth for the last 10-12 hours?	☐	☐
Medical/Oral History Was the medical/oral history updated?	☐	☐
Oral Soft Tissue Exam Were photographs taken?	☐	☐
Was an oral soft tissue exam performed?	☐	☐
Whole Saliva Was whole saliva collected?	☐	☐
_____ ml collected in _____ minutes (max. 15)		
Plaque Index Was the Plaque Index (PI) completed?	☐	☐
The mean PI was _____		
Gingival Index Was the Gingival Index (GI) completed?	☐	☐
The mean GI was _____		

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

	Yes	No
Gingival Crevicular Fluid (Pre-brushing) Was a gingival crevicular fluid sample collected pre-brushing?	☐	☐
Which teeth were sampled? ____ ____ ____ ____		
Plaque Biofilm Were sub-gingival plaque samples collected?	☐	☐
Which teeth were sampled? ____ ____ ____ ____		
Gingival Crevicular Fluid (Post-brushing) Was a gingival crevicular fluid sample collected from the same teeth 1 hour post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 2 hours post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 4 hours post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 6 hours post-brushing?	☐	☐
Oral Hygiene Was the subject instructed to continue oral hygiene practices?	☐	☐
Payment Was the subject given $100 compensation?	☐	☐

_____ _____

Investigator Signature Date

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

PLAQUE INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean PLAQUE INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria
0	No plaque
1	A film of plaque adhering to free gingival margin and adjacent area of tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

GINGIVAL INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
GI																					
	18			**19**			**20**			**21**			**22**			**23**			**24**		
GI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
	31			**30**			**29**			**28**			**27**			**26**			**25**		
GI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean GINGIVAL INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria	Bleeding
0	Absence of inflammation	No bleeding on probing
1	Mild inflammation Slight change in color and texture	No bleeding on probing
2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

APPENDIX M

Day 21 Examination Form

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (if "Yes" complete AE form)	**YES**	**NO**

	Yes	**No**
Tooth Brushing Has the subject refrained from brushing their teeth for the last 10-12 hours?		
Medical/Oral History Was the medical/oral history updated?		
Oral Soft Tissue Exam Were photographs taken?		
Was an oral soft tissue exam performed?		
Whole Saliva Was whole saliva collected? _____ ml collected in _____ minutes (max. 15)		
Plaque Index Was the Plaque Index (PI) completed? The mean PI was _____		
Gingival Index Was the Gingival Index (GI) completed? The mean GI was _____		
Gingival Crevicular Fluid (Pre-brushing) Was sub-gingival plaque samples collected pre-brushing? Which teeth were sampled? ____ ____ ____ ____		

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

	Yes	No
Plaque Biofilm Were sub-gingival plaque samples collected?	☐	☐
Which teeth were sampled? ____ ____ ____ ____		
Gingival Crevicular Fluid (Post-brushing) Was a gingival crevicular fluid sample collected from the same teeth 1 hour post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 2 hours post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 4 hours post-brushing?	☐	☐
Was a gingival crevicular fluid sample collected from the same teeth 6 hours post-brushing?	☐	☐
Pr ophylaxis Did the subject receive a full-mouth prophylaxis?	☐	☐
Was subject given OHI?	☐	☐
Compliance Were all stents collected?	☐	☐
Was remaining product collected (A quantity of more than 60% remaining will indicate lack of study compliance and grounds for dismissal from the study)?	☐	☐
Payment Was the subject given $100 compensation?	☐	☐

_____ _____

Investigator Signature Date

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

PLAQUE INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
PI																					
	2			**3**			**4**			**5**			**6**			**7**			**8**		
PI																					
LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**

LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
PI																					
	15			**14**			**13**			**12**			**11**			**10**			**9**		
PI																					
BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean PLAQUE INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria
0	No plaque
1	A film of plaque adhering to free gingival margin and adjacent area of tooth. The plaque may be seen *in situ* only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

GINGIVAL INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
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BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
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Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

Mean GINGIVAL INDEX for Patient:

Total ÷ Number of Sites = MEAN

_____ ÷ _____ = _____

Scores	Criteria	Bleeding
0	Absence of inflammation	No bleeding on probing
1	Mild inflammation Slight change in color and texture	No bleeding on probing
2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

APPENDIX N

Day 35 Examination Form

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

ADVERSE EVENTS		
Have there been any adverse events? (if "Yes" complete AE form)	**YES**	**NO**

Tooth Brushing

Has the subject refrained from brushing their teeth for the last 10-12 hours? ☐ Yes ☐ No

Medical/Oral History

Was the medical/oral history updated? ☐ Yes ☐ No

Oral Soft Tissue Exam

Were photographs taken? ☐ Yes ☐ No

Was an oral soft tissue exam performed? ☐ Yes ☐ No

Whole Saliva

Was whole saliva collected? ☐ Yes ☐ No

_____ ml collected in _____ minutes (max. 15)

Plaque Index

Was the Plaque Index (PI) completed? ☐ Yes ☐ No

The mean PI was _____

Gingival Index

Was the Gingival Index (GI) completed? ☐ Yes ☐ No

The mean GI was _____

Gingival Crevicular Fluid

Was a gingival crevicular fluid sample collected? ☐ Yes ☐ No

Which teeth were sampled? ____ ____ ____ ____

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MON/DD/YY) / /	**Patient Number**	**Patient Initials**

Plaque Biofilm

Were sub-gingival plaque samples collected? ☐ Yes ☐ No

Which teeth were sampled? ____ ____ ____ ____

Payment

Was the subject given $300 compensation? ☐ ☐ No

_____ _____

Investigator Signature **Date**

Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

PLAQUE INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
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BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
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Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

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Scores	Criteria
0	No plaque
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Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

GINGIVAL INDEX

BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**
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LINGUAL	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**	**d**	**l**	**m**
GI																					
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BUCCAL	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**	**d**	**b**	**m**

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Experimental Gingivitis Model for the Study of Anti-Inflammatory Effects of Colgate Total® - a Pilot Investigation			
University of Michigan	**Date** (MM / DD / YY) / /	**Patient Number**	**Patient Initials**

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2	Moderate inflammation, glazing, redness, edema and hypertrophy	Bleeding on probing
3	Severe inflammation, redness and hypertrophy. Ulceration.	Tendency to spontaneous bleeding

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