CLINICAL EVALUATION OF BIOFILM CONTENT IN ORTHODONTIC PATIENTS TREATED WITH PROPHY-JET™ VERSUS CONVENTIONAL HOME CARE

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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I would like to thank my mother and father for their never ending support.

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Orthodontic appliances can accumulate plaque, leading to gingivitis, enamel decalcification, and dental decay. Currently, plaque removal in the orthodontic office is limited to tooth-brushing after wire removal. A prospective randomized clinical trial was conducted to compare the Prophy-Jet™ to manual tooth-brushing for removing dental plaque and effecting bacterial content of plaque.

Our study included 24 orthodontic patients with fixed appliances showing poor oral hygiene were recruited to participate in the study for 7 consecutive orthodontic appointments (T_0-T_6). The study group consisted of 17 males and 7 females with an average age of 14.1 years (range 10 to 17). Subjects were randomly assigned to two groups. The first group had their teeth cleaned each visit with the Prophy-Jet™ by a dental professional; the second group brushed their own teeth with a manual tooth brush at each visit. Plaque Index (PI), Papillary Bleeding Score (PBS), and Decalcification Index (DI) were assessed throughout the study. In addition to the indices, plaque samples were taken for analysis of the bacterial content by DNA-DNA hybridization.

There was a significantly lower mean change in PI score for the prophy-jet group compared to the toothbrush group comparing baseline to time point 6 (prophy-jet mean change -0.42, toothbrush mean change 0.20, positive change indicates improvement). The bacteria species present in the plaque samples remained fairly constant. The samples tested positive for the same bacteria at different time points and different patients. The Prophy-Jet™ had no effect on plaque content. Cleanings with the Prophy-Jet™ at each orthodontic visit were not effective in changing the PI, DI, PBS or bacterial content of orthodontic patients with poor oral hygiene when compared to conventional tooth brushing.

CHAPTER 1
INTRODUCTION

Dental biofilm has long been associated with tooth decay and periodontal disease. Fixed orthodontic appliances, specifically brackets bonded to teeth, can accumulate plaque, and may interfere with effective plaque removal. Proper oral hygiene by patients undergoing orthodontic treatment, is usually difficult to maintain. Accumulated dental plaque in orthodontic patients has been associated with enamel decalcification, enamel scarring, dental decay, and gingivitis.

Highly aciduric bacteria such as Streptococcus mutans and lactobacilli have been reported following the placement of orthodontic appliances.[1] These microorganisms produce acid as a metabolic byproduct lowering the intraoral pH, and increasing the risk of decalcification or white spot formation.[2] In a study of decalcification incidence, 50% of orthodontic patients experienced an increase in decalcification during treatment, with the highest incidence in the maxillary incisor region and lowest incidence in the maxillary posterior region.[3]

In addition to decalcification, several studies have also shown strong correlations between the microbial composition of subgingival biofilms and periodontal disease, most notably species such as P. *gingivalis* and A. *actinomycetemcomitans.* Aa has been associated with aggressive periodontitis.[4] Although methodologies for characterizing the microbial flora in a dental biofilm vary greatly, the importance of elucidating the bacterial species that may be responsible for causing a wide range of oral health problems, ranging from periodontal disease to dental caries, cannot be exaggerated; especially considering recent research implicating periodontal disease as a risk factor for cardiovascular complications, low term birth weight, and diabetes.

Since its introduction in 1977, air-powder polishing systems have been effective at removing stain and plaque.[5] The design of the various air-powder polishing systems, such as Dentsply's Prophy-Jet™, use a mixture of air, water, and sodium bicarbonate to deliver a

controlled stream of sodium bicarbonate particles to the tooth surface. Advantages of air polishers include rapid removal of tooth deposits, less invoked hypersensitivity;[6,7] less operator fatigue;[8] and improved access to pits and fissures.[9] Currently, plaque removal in the orthodontic office is selectively limited to tooth-brushing after wire removal. This method of plaque removal requiring wire removal relies on the patient's ability to effectively remove the plaque with a manual tooth-brush. The Prophy-Jet™ has been shown to be effective in general dental patients during supportive periodontal therapy, and to decrease decalcification in orthodontic patients.[10] Its effect on the microorganisms within plaque has not been studied.

The aims of this clinical trial were to evaluate the long-term effects of monthly debridements with the Prophy-Jet™ in orthodontic patients with poor oral hygiene on gingivitis, decalcification, and plaque accumulation; to evaluate the effect on biofilm content with the Prophy-Jet™ in orthodontic patients with poor oral hygiene using the DNA-DNA hybridization technique; to compare the effectiveness of the Prophy-Jet™ to currently used method of manual tooth-brushing.

CHAPTER 2
METERIALS AND METHODS

The study was designed as a prospective randomized controlled clinical trial. Patients undergoing orthodontic treatment with brackets and wires in the maxillary and mandibular arches between the ages of 10 and 18 were recruited from the Graduate Orthodontic Clinic at the University of Florida, College of Dentistry. Other selection criteria for inclusion in the trial were the presence of extensive amounts of visually detectable plaque around the orthodontic appliances as identified by the operator, good health with no current medications, and willingness to sign informed consent. Participants agreed to continue their normal daily home care routine, and refrain from professional cleanings during the study. The Institutional Review Board for research at the University of Florida approved the protocol prior to starting the study.

The subjects were randomly assigned to two groups by a computer-generated sequence. The first group had their teeth cleaned with the Prophy-Jet™ by a dental professional; the second group brushed their own teeth with a manual tooth brush at each visit. For participation in the trial, subjects were financially compensated and received a full mouth debridement with the Prophy-Jet™ at completion of the study.

The protocol for each clinical visit was designed based on a previous study that was conducted in 2003 at the University of Florida.[10] Participants were seen for a total of seven clinical visits, the first consisting of a baseline examination of dental health parameters including the collection of clinical indices, and an initial plaque sample. At this initial visit, a Prophy-Jet™ cleaning was completed by a dental professional or a manual tooth-brushing by the subject. Also, a plaque sample was taken from each subject. The right maxillary and mandubular lateral incisors and first premolars were sampled by carefully moving an explorer between the orthodontic bracket and gingival margin. Samples were stored at -80˚C in 1ml of TE buffer

solution for DNA-DNA hybridization analysis at the completion of the study. Subjects were then seen at regular orthodontic appointments for 7 visits, at which times clinical indices were recorded and plaque samples collected.

The labial surfaces of teeth bonded with brackets from the 2nd premolars forward were included in this assessment. The following clinical indices were measured for this study.

Plaque levels were assessed from 0 to 5 using the Turesky modification of the Quigley-Hein Plaque Index (PI).[11] The PI was recorded at each visit before the Prophy-Jet™ or toothbrush cleanings were completed.

Decalcifications were assessed visually and tactilely with a dental explorer and scored from 0 to 4 by using a modified version of the white spot lesion index of Gorelick et al.[3] The decalcification index (DI) was measured at baseline, time point 3, and time point 6. Decalcification assessment was recorded after removal of plaque to allow complete unobstructed visualization of the enamel.

Gingivitis was assessed at each visit with a score of 1 to 5 using the Papillary Bleeding Score (PBS) of Loesche.[12] A Stimu-dent™ was used to stimulate the interdental papilla, which is a common site of gingival inflammation for orthodontic patients. Subsequent gingival bleeding was used as a measure of gingival health.

A single operator performed all measurements and cleanings. Before recruitment of subjects for the Prophy-Jet™ study, the operator was calibrated by a calibrated by a standardized examiner on the three clinical indices (DI, PI, and PBS). Use of the Prophy-Jet™ was also calibrated to ensure consistency. Five orthodontic patients meeting the inclusion criteria of the planned study were examined by operator and hygienist and standardization and reproducibility of indices were demonstrated. These patients were not included in the clinical trial.

Twenty-four orthodontic patients with fixed appliances showing poor oral hygiene were recruited to participate in the study. The study group consisted of 17 males and 7 females with an average age of 14.1 years (range 10 to 17). Over the course of the study, 8 patients were eliminated for missing appointments or discontinuing orthodontic treatment, leaving a total of 16 patients to complete the study.

Collected plaque samples were stored in 1ml of TE buffer (10 mM Tris-HCL, 1mM EDTA, pH 7.6) at -80˚C and analyzed for bacteria content using checkerboard DNA-DNA hybridization. The process originally described by Socransky and Wall-Manning was followed for the analysis.[13,14,15] However, the BrightStar system (Ambion, Inc., Austin TX) was substituted for the digoxigenin-labeling system. Table 2-1 shows a list of bacterial strains used for probes in the analysis.

Table 2-1. Bacteria analysed by DNA-DNA hybridization

Streptococcus gordonii*	Actinomyces isralii
Streptococcus mutans	Actinomyces naeslundii*
Streptococcus oralis*	Actinomyces odontolyticus*
Streptococcus salvarius	Actinomyces viscosus
Prevatella intermedia*	Porphyromonas gingivalis*
Prevatella oralis*	Lactobacillus casei
Fusabacterium nucleatum*	Lactobacillus rhamosus
Veillonella parvula*	Tannerella forsythensis*
Actinobacillus actinomycetemcomitans*	

* Bacteria present in the plaque samples

CHAPTER 3
STATISTICAL METHOD

Changes in mean scores that occurred during treatment for PI, DI, and PBS were evaluated with the 2-sample t-test, as well as the Wilcox rank sum test. The presence or absence of bacteria in the plaque samples were not statically evaluated.

CHAPTER 4
RESULTS

Twenty-four orthodontic patients with fixed appliances showing poor oral hygiene were recruited to participate in a study comparing the Prophy-Jet™ to manual tooth brushing. The study group consisted of 17 males and 7 females with an average age of 14.1 years (range 10 to 17). Over the course of the study, 8 patients were eliminated for missing appointments or discontinuing orthodontic treatment, leaving a total of 16 patients to complete the study. The 16 subjects consisted of 11 males, 5 females, 8 in the Prophy-Jet™ group and 8 in the toothbrush group. At baseline (T_0) there were no statistical differences between the two groups for PI, DI, or PBS. The low number of subjects (16) did not provide adequate power to detect clinically significant differences.

The change in mean PI scores in the maxillary arch between the Prophy-Jet™ and toothbrush groups was statistically significant comparing baseline to time point 6 (prophy-jet mean change -0.42, toothbrush mean change 0.20, positive change indicates improvement). The Prophy-Jet™ subjects demonstrated a greater PI score with significance levels of $p=0.05$ at T_6. No statistical significance for PI score was found in the mandibular arch (Figure 4-1). A comparison of the cleaning effectiveness between the Prophy-Jet™ and manual tooth-brushing for each visit is illustrated in Figure 4-1.

The change between baseline and time point 6 in mean decalcification scores (Figure 4-2) between the Prophy-Jet™ and toothbrush was borderline statistically significant ($p=0.06$) (prophy-jet mean change -0.11, toothbrush mean change -0.01, negative change indicates worsening) in the maxillary arch only. Thus, the prophy-jet group had a greater increase in decalcification score with a significance level of $p = 0.06$ at T_6. No statistical significance for decalcification score was found in the mandibular arch (Figure 4-2)

Although there was a trend of decreasing papillary bleeding scores over time, these were not statistical significance for in either arch (Figure 4-3).

Results of the DNA-DNA hybridization showed little to no change between patients in the two groups or between time points. The plaque samples consistently tested positive for the bacteria highlighted in Table 2-1. Intensity of staining was scored on a scale of 0-3 and equated to approximately 10^5 to 10^7 cells. Bacteria present, decalcification presence, total treatment time and treatment group data were cross referenced and examined for any correlations. Time intervals between subject visits are presented in Table 4-1. Inspection of these data showed no relationship between the amount of time, presence or absence of decalcification, presence or absence of certain bacteria and treatment group.

Table 4-1. Number of days between appointments for each patient

Patient No.	T0	T1	T2	T3	T4	T5	T6	Total
1	0	35	38	42	33	22	45	215
3	0	49	28	24	32	43	57	233
4	0	21	41	84	33	70	89	338
5	0	45	28	56	51	59	67	306
6	0	34	26	33	44	21	34	192
7	0	27	37	34	55	26	33	212
8	0	64	27	43	40	34	27	235
9	0	27	37	34	28	54	49	229
10	0	45	34	34	57	34	41	245
12	0	27	37	27	49	42	54	236
14	0	37	34	28	35	34	31	199
19	0	41	28	42	27	29	27	194
21	0	35	14	33	28	34	28	172
22	0	28	33	40	19	48	30	198
23	0	42	43	34	55	42	40	256
24	0	42	43	34	55	42	40	256

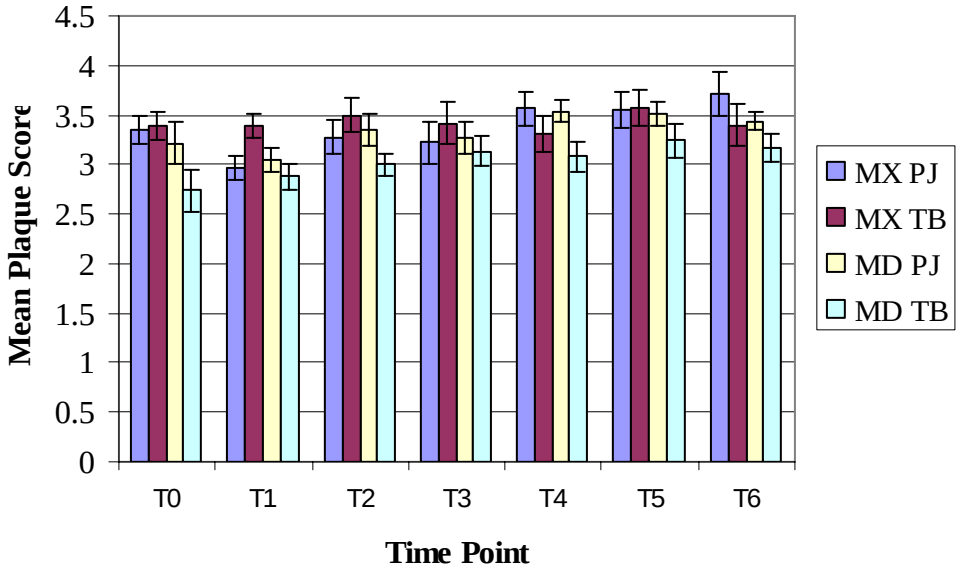

Figure 4-1. Plaque Index Scores (mean ± SE, n = 16). MX PJ = Maxillary Prophy-Jet, MX TB = Maxillary Toothbrush, MD PJ = Mandibular Prophy-Jet, MD TB = Mandibular Toothbrush

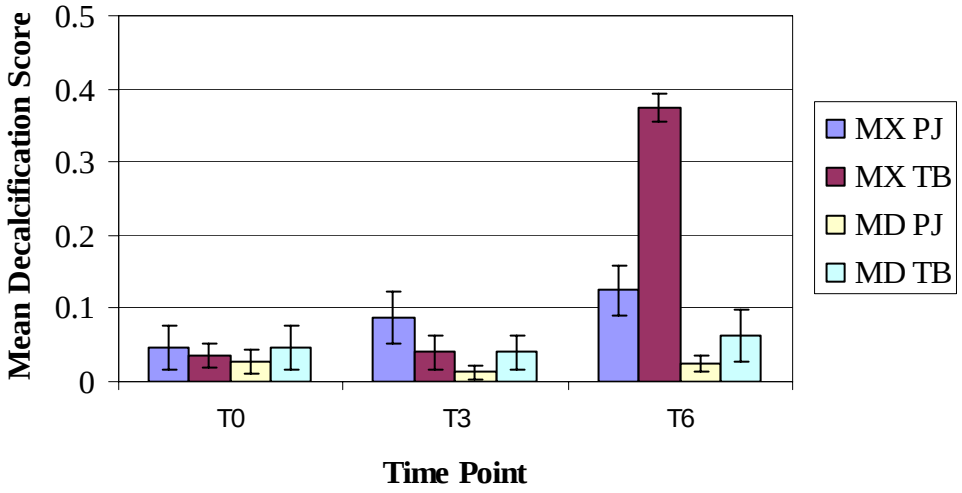

Figure 4-2. Decalcification Index Scores (mean ± SE, n = 16). MX PJ = Maxillary Prophy-Jet, MX TB = Maxillary Toothbrush, MD PJ = Mandibular Prophy-Jet, MD TB = Mandibular Toothbrush

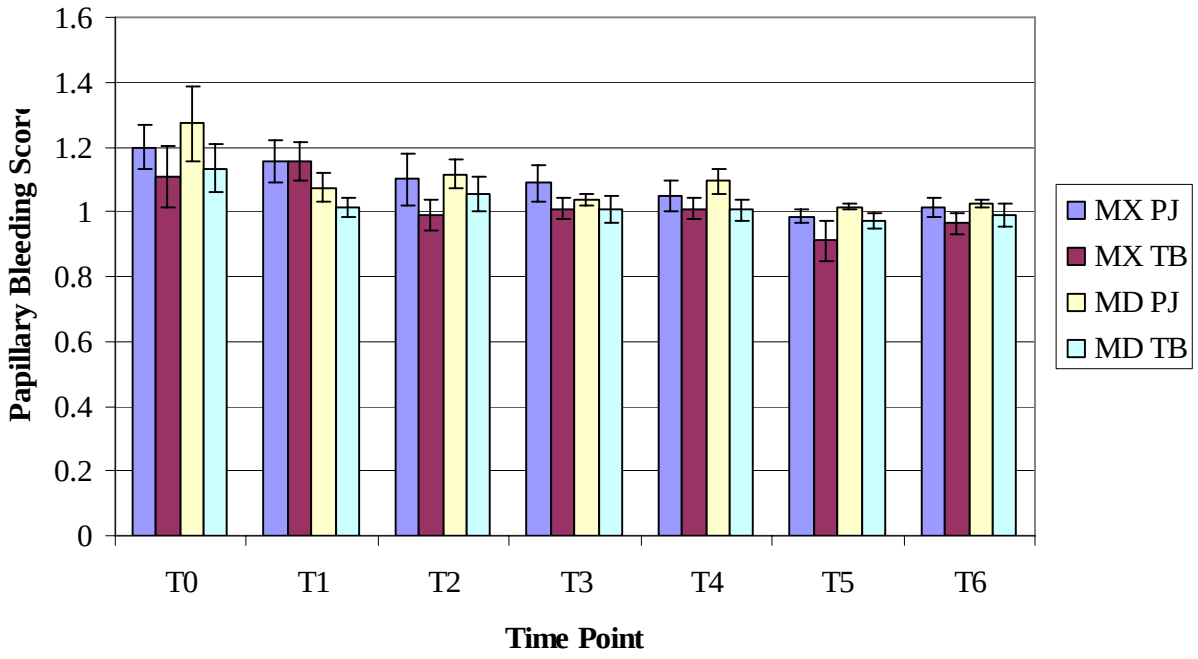

Figure 4-3. Papillary Bleeding Score (mean ± SE, n = 16). MX PJ = Maxillary Prophy-Jet, MX TB = Maxillary Toothbrush, MD PJ = Mandibular Prophy-Jet, MD TB = Mandibular Toothbrush

CHAPTER 5
DISCUSSION

The present study was conducted as a prospective randomized controlled clinical trial on 16 orthodontic patients with full fixed appliances demonstrating visible supragingival plaque. By selecting patients in orthodontic treatment with poor oral hygiene, this study attempted to determine whether regular Prophy-Jet™ cleanings would minimize the harmful effects of plaque thereby reducing any negative effects.

A study by Barnes and Gerbo et al.[16] investigated the application the Prophy-Jet™ device in an orthodontic setting. The study showed that the Prophy-Jet™ was more effective in removing plaque around orthodontic appliances; and required less time than traditional rubber cup/pumice prophylaxis.

In the present study, the outcomes of plaque debridement at regular orthodontic visits showed no difference between the Prophy-Jet™ and toothbrush groups for PI, although at time point 6, the toothbrush group had statistically significant lower plaque scores (Figure 1). PI scores were taken at the beginning of each appointment after the patient rinsed with disclosing solution. It can be speculated that the amount of plaque accumulation was closely related to the time interval since the patient last brushed his or her teeth. Patients seen during morning appointments seemed to have much less plaque accumulated as compared to afternoon appointments where the patient had eaten lunch and gone all day without brushing. Therefore, PI scores could vary greatly depending on appointment time. For future studies, plaque samples should be taken at the same time each day to prevent this confounding problem.

Another possible reason for the lack of improvement by the Prophy-Jet™ cleanings on plaque levels, gingival inflammation, and decalcification may be explained by biofilm formation and the study design. The salivary pellicle begins to form within minutes to hours after

professional dental cleaning and the initial colonizers are gram positive bacteria such as *Streptococcus* and *Actinomyces* species.[13] Over the following days, the plaque increases in thickness and quantity as gram-negative cocci and gram-positive and gram-negative rods and filaments increase their presence. The subjects included in this study were identified as having poor oral hygiene and while the duration of time between appointments apparently was enough time for plaque levels and gingivitis to return to baseline levels, it might be possible that if these time intervals were closer, a beneficial reduction might be seen. Any potential benefits of a professional cleaning would have to be maintained by the patients in their home care in order to minimize the damaging effects of plaque accumulation on the teeth and surrounding periodontium. However, poor oral hygiene patients are the ones that would most benefit from additional hygiene measures and might need more frequent intervention.

Oral hygiene maintenance by the subject appears to be very important in decreasing the time interval for bacteria to recolonize. A 4-week study completed by Ho et al. [17] compared the Sonicare toothbrush to a manual toothbrush. The subjects had full fixed orthodontic appliances and were instructed to brush every morning and evening for 2 minutes. The results showed statistically lower scores for plaque index, gingival index, and sites which bled on probing for the Sonicare group. Also, there was a decrease in the amount of total gram-negative bacteria in subgingival plaque samples in the same group. This may indicate that more frequent cleaning with the Prophy-Jet™ and reinforcing home care could increase oral health of the patients.

This study was patterned after a clinical trial completed by Albert et al. 2003, where regular cleanings with the Prophy-Jet™ were shown to decrease decalcification in orthodontic subjects.[10] Albert used a split mouth design, in which one side of the mouth was cleaned with the Prophy-Jet™ and the other side was cleaned by manual tooth brushing. Possibilities for a

decrease in the decalcification index include interference in the colonization of certain bacteria into the developing plaque on the tooth surface. Although there are numerous species of bacteria in plaque, most are noncariogenic.[18] Primary etiologic bacteria in dental caries include *Streptococcus mutans* and *Streptococcus sobrinus* with *Lactobacilli* as a secondary invader involved in caries progression in enamel.[19] Thus, even in the presence of unaltered plaque levels and gingivitis, the monthly debridement by the Prophy-Jet™ may have altered the bacterial load by physical disruption and prevented more harmful organisms from colonizing and promoting the decalcification process. The current study did not reach statistical significance which may have been due to the limited number of subjects.

Plaque content showed little change in volume or species present over the course of the study It was of interest that although the samples were taken from supragingival areas (between the bracket and gingival margin) the plaque resembled mature subgingival samples.[20] Instead of showing large numbers of Streptococcus mutans and Lactobacillus species, there were large amounts of Actinomyces *odontolyticus*, Actinobacillus *actinomycetemcomitans*, Tannerella *forsythensis*, Veillonella *parvula*, and Streptococcus *oralis* indicative of subgingival flora. This could be a product of the environment created by the bracket/tooth interface. The bracket mimics an overhanging restoration where bacteria can conlonize and mature in an anaerobic environment similar to subgingival tooth surfaces. It has been observed that dental restorations and fixed orthodontics increase the amount of plaque around teeth and cause more inflammation.[29] A study comparing the subgingival microbial composition 3 months after bracket placement showed an increase in P. *gingivalis*, P. *intermedia*, and T. *forsythia* in orthodontic subjects.[21] The current study displayed these bacteria in the supragingival plaque samples indicating that conditions mimicked the a subgingival environment. In addition, another study of microbial

flora on metal and ceramic brackets showed the presence of A. odontolyticus and Aa.[22] Both types of bacteria were present in large amounts in our study. Even though we did not observe an increase in decalcification in these subjects, it is possible with more time or an increase in subject number that these differences would be observed.

The volume of bacteria could have an important role in health tissue. Cleaning has been shown to reduce the bacterial count in patients and a subsequent decrease in both gingival index score and plaque index.[23] In this study, plaque was collected inconsistently by scraping 4 designated teeth. One to three swipes with an explorer was use to collect a generous amount of plaque possible. If the gingiva was hyperplastic and encroached on the bracket, the sample could contain subgingival bacteria. This was common in the premolar area. A possible solution to this problem would be to collect plaque by swiping a paper point between the bracket and the gingiva. This may create a more consistent and reliable result. In addition, plaque volumes could be quantified using new digital subtraction techniques.

Another problem encountered was that the DNA hybridization technique used whole genomic DNA with which cross reactions commonly occur. For example, pure DNA was run against the probes Pg which also cross reacted with other bacteria species. On the other hand, Aa was present in all subjects and did not cross react. This means Pg may or may not have been present in the samples, but Aa was definitely present in all samples. The presence of Aa is consistent with a study that reported 80% of young orthodontic patients are infected with Aa. The orthodontic patients showed more inflammation than non-orthodontic patients, but this did not necessarily correlate with the presence of Aa .[24]

Further limitations of the study include the small sample size which may not be representative of orthodontic patients as a whole. The low number of subjects was well under

the 100 subjects necessary to complete this study as determined by power analysis. Other potential limitations include geographic sampling bias, lack of blinding due to the single operator for decalcification index, effect of extraneous factors such as manual dexterity for the patients, and selection of clinical indices.

CHAPTER 6
CONCLUSION

Adequate home care is thought to be critical in maintaining oral hygiene and preventing decalcifications. For the present study, the Prophy-Jet™ proved to be unsuccessful in maintaining oral health or changing the biofilm content in orthodontic patients. Further research is necessary with an adequate patient population to further study this issue.

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BIOGRAPHICAL SKETCH

Michael Sutton was born and raised in Allentown, Pennsylvania. He received his degree of Bachelor of Science in civil engineering and a minor in environmental engineering at the Pennsylvania State University in 1997. In 2004, he earned a degree of Doctor of Dental Medicine from the Temple University School of Dentistry. Upon completion of his dental training, he continued his education at the University of Florida, College of Dentistry, receiving a certificate in orthodontics and Master of Science in 2007.