Original Article

A visual evaluation of oral plaque removal utilizing an adjunct enzyme pre-rinse in orthodontic subjects

Jennifer Rose[a]; Ahmed Ghoneima[b]; Frank Lippert[c]; Lisa Maxwell[d]; George Eckert[e]; Kelton T. Stewart[f]

ABSTRACT

Objective: To determine if an adjunct proteolytic pre-rinse along with contemporary methods of dental cleaning may more effectively remove visual plaque in subjects with fixed orthodontic appliances.

Materials and Methods: Forty-three orthodontic subjects, ages 10 to 25, completed this single site, double-blind, crossover clinical trial. Subjects randomly received bromelain enzyme or a powdered-sugar placebo pre-rinse, followed by manual tooth brushing and use of a Waterpik. Subjects received the alternate pre-rinse during the subsequent visit. Baseline and residual plaque accumulation were recorded via disclosing tablet and digital photography. A single, blinded examiner scored visual plaque scores from randomized photographs. Treatment effects on composite plaque score were evaluated using repeated-measures analysis of variance. A 5% significance level was used for all tests.

Results: No significant differences in plaque scores were noted at baseline or post-rinse between the enzyme and placebo. The changes from baseline to post-rinse ($P = .190$), post-brushing ($P = .764$), and post-Waterpik ($P = .882$) were not significantly different between interventions. Significant reduction in plaque scores were observed in both arms of the study after brushing ($P < .01$) and waterjet use ($P < .01$). Neither age ($P = .220$) nor gender ($P = .449$) impacted plaque scores.

Conclusions: Use of a bromelain enzyme pre-rinse alone did not significantly enhance plaque removal. A significant reduction in retained plaque was observed with the application of brushing and or Waterpik. (*Angle Orthod.* 2020;90:844–850.)

KEY WORDS: Proteolytic enzyme; Bromelain; Oral plaque removal; Oral hygiene; Fixed orthodontic treatment

[a] Resident, Department of Orthodontics and Oral Facial Genetics, Indiana University School of Dentistry, Indianapolis, IN, USA.

[b] Associate Professor and Department Chair, Department of Orthodontics, Hamdan Bin Mohammed College of Dental Medicine (MBRU), Dubai, UAE.

[c] Associate Research Professor, Director of Remineralization Research Program, Oral Health Research Institute, Indianapolis, IN, USA.

[d] Director of Dental Hygiene & Clinical Assistant Professor, Indiana University School of Dentistry, Indianapolis, IN, USA.

[e] Biostatistician, Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN, USA.

[f] Associate Professor, Chair, & Program Director, Department of Orthodontics and Oral Facial Genetics, Indiana University School of Dentistry, Indianapolis, IN, USA.

Corresponding author: Dr Kelton T. Stewart, Indiana University School of Dentistry, Department of Orthodontics and Oral Facial Genetics, 1121 West Michigan Street, DS 206, Indianapolis, IN, 46202, USA
(e-mail: keltstew@iu.edu)

Accepted: June 2020. Submitted: December 2019.
Published Online: September 14, 2020

INTRODUCTION

Orthodontic appliances create obstacles to oral hygiene, resulting in increased plaque accumulation,[1] inadequate oral hygiene,[2] and orthodontic-induced gingivitis.[3,4] Residual plaque in orthodontic subjects is often found in challenging-to-reach areas (near the gum line, around brackets, and beneath wires).[1]

A paucity of literature exists regarding proteolytic hydrolysis in dental oral hygiene. Due to the innate proteolytic activity of enzymes, proteins are hydrolyzed, yielding peptides and amino acids. Enzymes have the potential to lyse adhesive bonds between bacteria and the dental pellicle, delaying bacteria from colonizing on tooth surfaces.[5] In vivo enzyme studies have demonstrated reductions in plaque and gingivitis.[6]

Bromelain, a naturally occurring cysteine protease derived from pineapple stalks, is used in food and medical industries and listed on the US Food and Drug Administration's "Generally Recognized As Safe" list.[7]

 DOI: 10.2319/120819-776.1

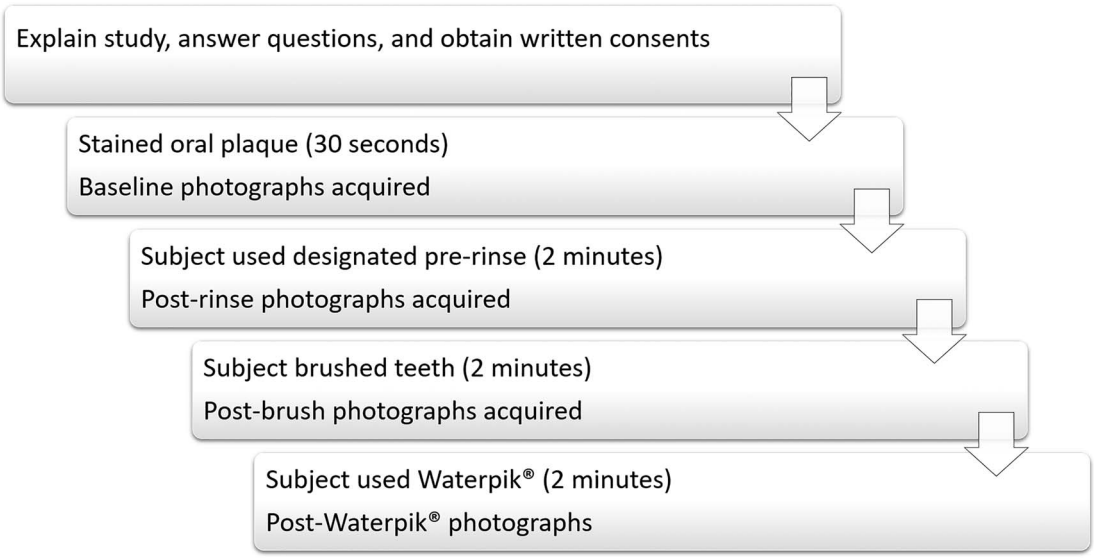


Figure 1. Diagram outlining the clinical procedure steps associated with the study.

An in vitro bromelain study demonstrated antibacterial effects on oral pathogens.[8] Bromelain was found to prevent biofilm formation by interfering with bacteria-bacteria adhesion and or adhesion to the enamel surface.[9] Clinical trials with toothpaste containing bromelain demonstrated improved plaque and gingivitis scores[10] and extrinsic enamel stain removal capabilities.[11]

The aim of this study was to investigate the impact of a bromelain pre-rinse adjunct on oral plaque removal in orthodontic subjects. The null hypothesis was that there would be no statistically significant difference in visual plaque scores with or without the use of a proteolytic enzyme rinse aid.

MATERIALS AND METHODS

This randomized, single site, crossover, double-blind clinical trial was approved by the Indiana University-Purdue University Institutional Review Board (IRB protocol number: 1802369383). Forty-six (46) subjects were recruited from the Indiana University School of Dentistry, Department of Orthodontics and Oral Facial Genetics clinic. Subjects recruited for the study were existing patients in active treatment, who had fixed metal twin bracket appliances placed prior to enrollment. No attempt was made to standardize patients with one particular bracket type or slot size. Recruited subjects had both 0.018" and 0.022" brackets manufactured by three companies: 3M, American, and ORMCO. Additionally, the subjects recruited were at various stages of active orthodontic treatment. All required treatment consents were obtained from the subjects and or their legal guardians prior to participation in the study. Investigators reviewed subject medical histories to ensure all subjects possessed good health and were fit for participation. Oral soft and hard tissue exams were performed at study visits to ensure subject safety. Subjects were asked to abstain from oral hygiene procedures the morning of and day prior to study visits. A washout period between interventions (1 week $\pm$ 2 days) ensured adequate baseline plaque levels were present. At the subsequent visit, subjects performed the same protocol, but received the converse test rinse. Data acquisition took place over 1 month. An overview of the clinical protocol is illustrated in Figure 1.

Inclusion criteria were: male or female; 10 to25 years of age; willing to consent to treatment; able to follow instructions; and undergoing active orthodontic treatment with fixed metal twin orthodontic appliances. Exclusion criteria were: unwilling/unable to follow study instructions; documented/suspected pineapple allergy; proteolytic enzymes allergy; food dye allergy; and or smoker.

One gram of the enzyme (bromelain) or control (powdered sugar) was weighed on a digital scale (Uniweigh, Shanghai, China), placed into an individual 20-mL vial, and sealed with a plastic cap to prevent contamination and or spillage. Vials were wrapped with paper to conceal the contents. After vial preparation was complete, a neutral individual worked with the biostatistician to code the vials in preparation for the randomized stratification process. After the preparation and coding process was complete, the investigators were blinded and unaware of the vial content or sequence of vial distribution. The bromelain enzyme was obtained in bulk from Ultra Bio-logics Inc. (Chateauguay, Quebec, Canada). The amount of enzyme used in this study was in accordance with

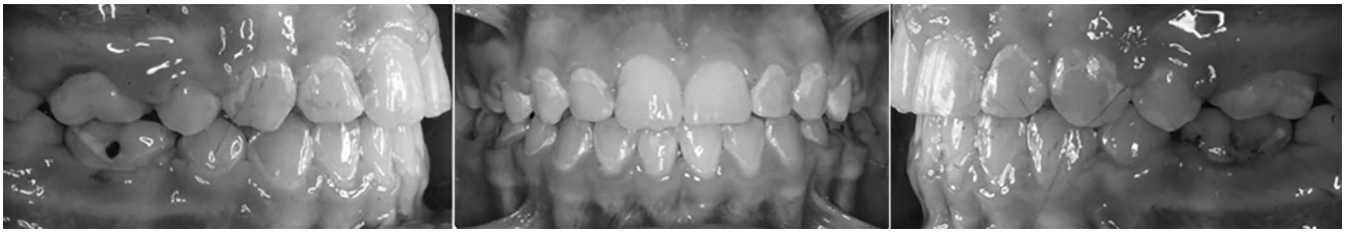


Figure 2. Photographic image series collected after each stage throughout the study (right buccal, frontal, and left buccal).

the manufacturer's recommendation for dietary oral consumption. Bromelain is stable at room temperature in dry powder form but active in liquid form. The dry powders were reconstituted chairside with 15 mL of warm pineapple juice just prior to administration. A 5" disposable swizzle straw (AmerCareRoyal, Oakville, Ontario, Canada) was used to stir the solution. Canned pineapple juice (Dole Food Company, Westlake Village, CA) was used since the canning manufacturing process destroys the active form of bromelain, while maintaining an optimal enzymatic pH for reconstituting the powder. This ensured a standard quantity of enzyme for all subjects. Bromelain's optimal activity is 55°C, so a temperature-controlled hot plate warmed an electric kettle of pineapple juice to 50°C.

All protocol steps were timed (Figure 1) with a digital timer (Sunbeam Products Inc., Boca Raton, FL) for consistency. The same brand of C-shaped cheek retractors, size L (EZGO, Ontario, Canada) and digital camera (Pentax K-50 digital camera, Ricoh, Tokyo, Japan) with Lester Dine 105-mm F/2.8 macro lens (Dine Corp., Palm Beach Gardens, FL) and 52-mm Eitar SER-VII UV filter (Eitar, Japan) were used for all subjects. The same camera settings (*f*-stop: 32, shutter speed: 1/60, ISO: 200) were used for all images. The camera was mounted on a tripod and photos were captured under the same clinical conditions. All subjects used the same brand of pre-pasted, individually wrapped, soft bristle, toothbrush: Vivid Premium Quality Toothbrush (Pearson Dental Supply Co, Sylmar, CA), Waterpik: Aquarius Professional Designer Series (Waterpik Inc., Fort Collins, CO), and Two Tone chewable disclosing tablets (TePe USA, Anaheim, CA). Subjects received a new toothbrush and disclosing tablet at each visit.

Intraoral photographs were acquired of every subject at each treatment step to document plaque scores. The photographic series included one frontal and two buccal images of the dentition (Figure 2). One investigator (JR) reviewed all photographs immediately after acquisition to ensure acceptable diagnostic quality.

The clinical procedure began with subjects chewing and swishing a disclosing tablet in their mouth for 30 seconds. They were then asked to expectorate prior to baseline image acquisition. Thereafter, subjects received the designated vial and were instructed to place the entire solution in their mouth and swish for 2 minutes. They expectorated the solution after the allotted time and had post-rinse photographs taken. Subjects then brushed with a provided toothbrush and were instructed to "brush your teeth as you normally would." After the attainment of post-brushing photographs, the subjects used the Waterpik and were instructed to "use this device (Waterpik) to the best of your ability to clean your teeth." The waterjet reservoir (600 mL) was filled with tap water at room temperature and a standard waterjet tip was used. Subjects were allowed to use a mirror, which was covered in pink tinted wrap (The Michaels Companies, Inc., Irving, TX) to obscure visualization of the disclosing agent on their teeth.

A single examiner from the Indiana University School of Dentistry, Department of Dental Hygiene assessed the subjects' degree of plaque retention via digital photographs. The examiner's reliability was calibrated by scoring 10 randomly selected images, which were re-scored after a 2-week washout period. Adequate reliability was assessed using intraclass correlation coefficients and a threshold of 0.8 was set for the study. Once the reliability threshold was reached, the examiner scored each photograph in the study. The coding scheme used to label the photographs did not allow the examiner to link a photograph with a specific subject, intervention, or stage. Each photograph was independently scored using the modified Orthodontic Plaque Index visual scale[12] (Figure 3). Plaque scores were assigned for the maxillary and mandibular incisors and a total score was obtained by summing the values of the eight teeth.

Statistical Analysis

Power analysis determined that a sample size of 45 subjects would possess 90% power to detect an effect size of 0.5 between the intervention and control legs of the crossover design. This prediction was based on a two-sided paired *t*-test conducted at a 5% significance level and assuming a correlation of 0.5 between the study legs. Although only 43 subjects completed the study, correlation between study legs was higher than

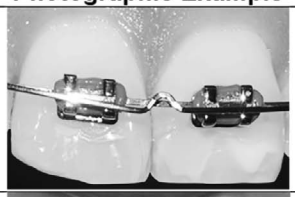
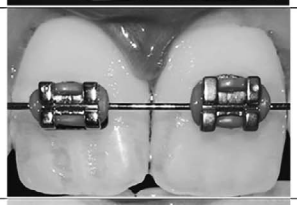
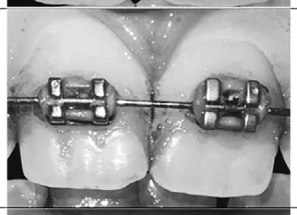
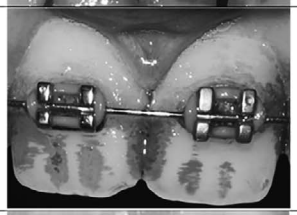
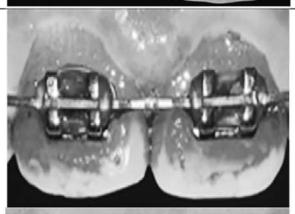
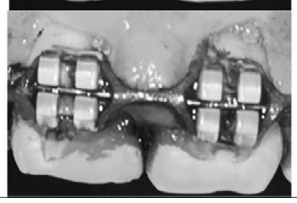
OPI score	Photographic Example	Description
0		Brackets are plaque-free.
1		Plaque one tooth surface at the bracket base.
2		Plaque on two surfaces at the bracket base.
3		Plaque on three tooth surfaces at the bracket base.
4	 	Plaque on all tooth surfaces at the bracket base; and/or gingival inflammation.

Figure 3. Orthodontic Plaque Index (OPI) visual scale used in the study, scored from 0 to 4.

expected (greater than 0.6 for the post-brushing and post-Waterpik plaque scores), so the study still possessed greater than 90% power to detect the planned effect size.

Intra-examiner repeatability of individual surface scores was evaluated using weighted kappa statistics and repeatability of subject-level index scores was evaluated using intraclass correlation coefficients (ICCs) and Bland-Altman plots. Acceptable intra-examiner repeatability was observed with ICC values greater than 0.80. Subject-level plaque scores were summarized by treatment (enzyme or placebo) and stage (baseline, post-rinse, post-brushing, and post-Waterpik).

The effects of treatment on composite plaque scores were evaluated using repeated-measures analysis of variance, which included fixed effects for treatment, stage, and their interaction. Stage was treated as a repeated factor within the subject for each study arm and included a factor for study arm. Age and gender groups were included as covariates, due to stratified randomization. In the stratified randomization protocol, each gender (male and female) was subdivided by age into two groups: less than 18 years of age and 18 years of age and older. Within the four groups, a number sequence was denoted that corresponded to the order in which subjects would receive the placebo and proteolytic enzyme pre-rinse. Statistical analyses were

Table 1. Paired *t*-Test Assessing Average Sum Plaque Scores

Intervention Stage	Enzyme Mean (SD)	Placebo Mean (SD)
Baseline	36.2 (10.7)	37.9 (10.8)
Post-rinse	34.8 (11.7)	37.8 (10.1)
Post-brush	11.4 (7.7)*	13.7 (7.4)*
Post-Waterpik	5.9 (5.7)*	7.9 (6.0)*

* $P < .05$.

performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

RESULTS

Forty-three subjects (23 female and 20 male) completed the study and were included in the statistical analysis. Mean subject age was 15.8 (SD = 2.6) and ranged from 11 to 22 years. A total of 53% (23 of 43) of the study subjects were female. Three consented subjects dropped out of the study. One subject elected not to participate after providing consent to participate but before the first data collection appointment. The other two subjects violated the established protocol (brushed their teeth on the morning of the second data collection appointment) and their complete data was excluded from statistical analysis. No subjects presented with or reported any discomfort during this study.

No significant differences were found between enzyme and placebo at baseline ($P = .406$) or post-rinse ($P = .151$) (Table 1). Baseline and post-rinse stages were not significantly different for enzyme ($P = .055$) or placebo ($P = .947$). The changes from baseline to post-rinse ($P = .190$), post-brushing ($P = .764$), and post-Waterpik ($P = .882$) were not significantly different between interventions. Enzyme application resulted in significantly lower composite plaque scores than placebo post-brushing ($P = .012$) and post-Waterpik ($P = .005$).

The plaque scores for all other stages were significantly different from each other for both interventions ($P < .001$): baseline and post-rinse > post-brushing > post-Waterpik. Stage impacted plaque score ($P < .001$), as there was a significant reduction of plaque in both arms of the study post-brushing and post-Waterpik. Subject gender ($P = .449$) and age ($P = .220$) did not significantly impact plaque scores (Table 2).

Table 2. ANOVA Comparisons of Different Effects on Plaque Scores[a]

Effect	Num DF	Den DF	*F* Value	*P* Value
Age	1	293	1.51	.220
Gender	1	293	0.58	.449
Treatment Sequence	1	293	0.81	.369
Arm	1	293	0.03	.873
Intervention	1	293	4.08	.044
Stage	3	293	310.17	<.001***
Intervention + Stage	3	293	0.62	.605

[a] ANOVA indicates analysis of variance. * $P = .05$; ** $P = .01$; *** $P = .001$; **** $P = .0001$. Num DF = degrees of freedom for the numerator; Den DF = degrees of freedom for the denominator

DISCUSSION

No significant differences were noted for plaque scores at baseline or post-rinse for either arm of intervention, which indicated there was no bias in data toward test rinse, either before or after rinsing, with or without enzyme. The bromelain pre-rinse alone did not significantly increase plaque removal.

The data demonstrate that, even under controlled settings, subject plaque removal was inadequate (only 64% plaque removal with manual tooth brushing alone). Similarly, van der Weijden and Slot found that manual tooth brushing removed an average of 42% of dental plaque.[13] Likewise, Atassi and Awartani found that 30% of orthodontic subjects had poor oral hygiene, with average plaque scores around 65%.[14]

Enzyme use alone did not yield a significant reduction in composite plaque scores. However, the data demonstrated a trend that suggests proteolytic enzymes could be a potentially beneficial adjunctive agent when combined with traditional forms of oral hygiene. Study results indicated a small benefit by including a proteolytic enzyme in the cleansing routine of orthodontic subjects. This was hypothesized to occur through a number of possible actions including bromelain enzymatically detaching plaque from the dental pellicle, the enzyme dissolving the protein components of the biomatrix of dental plaque, or both. It is likely that hydrolysis interfered with adhesion of the dental pellicle to the tooth surface, since the dental pellicle is made up of glycoproteins[15] and the enzyme acted to cleave protein bonds. In vivo and in vitro studies in the literature demonstrated that protease limits oral biofilm formation by digesting fimbriae and inhibiting biofilm formation.[16] Enzymes hydrolyze extracellular glucans, which thereby inhibit bacteria from adhering and forming plaques.[17]

Waterpik use after manual brushing significantly decreased residual plaque an average of 42%. The total combined effect of manual brushing and Waterpik use resulted in a 79% reduction of plaque. These findings were similar to other studies that found that use of pulsating water jet resulted in biofilm reduction[18] and decreased plaque,[4,19] more so than manual brushing alone.[19,20] With the addition of all three modalities, some subjects in the current study were able to completely remove all visual plaque.

The data demonstrated that gender and age did not affect dental plaque scores. This contradicts work by Kudirkaite et al., who found that older adolescent subjects with fixed orthodontic appliances had better

oral hygiene.[21] Unlike the current findings, Broadbent et al. determined that 15-year-old individuals had the highest average plaque scores.[22] Work by Atassi and Awartani observed no impact of gender on oral hygiene, which was similar to the results of the current study.[14] However, the current findings contrasted with those of Mei et al.,[23] who found that female adults had the least amount of dental biofilm.

The current study had a few acknowledged limitations. The sample size was slightly smaller than desired due to subject dropout. Additionally, a longer enzyme exposure time may have demonstrated effects that were more significant; however, such a change would have resulted in a protocol that was less clinically applicable. Additionally, the subjects recruited into the study had fixed metal twin brackets manufactured by different companies and with different slot sizes. The subtle variations in bracket size and configuration also could have served as a minor confounding factor for the current study.

Although this study did not find that a bromelain pre-rinse was independently effective in decreasing dental plaque, further studies with a larger sample size and additional standardization steps could demonstrate its utility as an oral hygiene adjunct. Additionally, the use of bacterial co-aggregation to evaluate the effectiveness of the proteolytic enzyme pre-rinse opposed to visual plaque evaluation may provide results that are more conclusive.

CONCLUSIONS

- Use of an oral bromelain pre-rinse alone did not increase dental plaque removal.
- Manual tooth brushing and Waterpik demonstrated significant plaque reduction.
- Further clinical studies are warranted to investigate the impact of enzyme pre-rinses on dental plaque removal and oral hygiene in orthodontic subjects.

ACKNOWLEDGMENTS

The authors thank Sue Kelly, Jennifer Tran, Keli Seering, Ultra Bio-logics Inc., the Delta Dental Master's Thesis Award program, and the Indiana University School of Dentistry Graduate Research Committee for their invaluable support of this project.

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