Elastomeric-ligated vs self-ligating appliances: A pilot study examining microbial colonization and white spot lesion formation after 1 year of orthodontic treatment

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Aim: *To (1) evaluate the use of adenosine triphosphate (ATP)–driven bioluminescence for quantification of total plaque bacteria in orthodontic patients, (2) compare plaque bacteria amounts at the bracket-tooth interface with use of elastomeric-ligated and self-ligating brackets after 1 year of orthodontic treatment, and (3) analyze formation of white spot lesions by photographic evaluation and laser-light fluorescence (DIAGNOdent).* ***Methods:*** *Thirteen subjects had fixed orthodontic appliances placed where lateral incisors were bonded with either elastomeric-ligated or self-ligating brackets. Plaque bacteria were collected from incisor surfaces after 1 year and quantified using plating methods and ATP-driven bioluminescence. White spot lesions were evaluated by photographic and DIAGNOdent determinations. A 2 × 2 × 2 mixed-design ANOVA was conducted to determine differences in plaque retention between elastomeric-ligated and self-ligating brackets.* **Results:** *ATP-driven bioluminescence values correlated to numbers of total plaque bacteria (r = 0.80). However, unlike findings published in the original pilot study, which described increased plaque retention with elastomeric-ligated brackets at 5 weeks postbonding, there were no significant differences in bacterial numbers or ATP-driven bioluminescence values surrounding the elastomeric-ligated vs self-ligating brackets after 1 year of orthodontic treatment. Based on photographic and DIAGNOdent determinations, white spot lesions were found relatively equally on teeth bonded with either bracket type. DIAGNOdent measurements were found to have moderate sensitivity (0.71) and good specificity (0.88) when compared to white spot lesions determined using photographic evaluation.* ***Conclusion:*** *ATP-driven bioluminescence can be used as an accurate assessment of total plaque bacteria in orthodontic patients. After 1 year of orthodontic treatment for patients in this pilot study, there appeared to be no differences in retention of plaque bacteria or white spot lesions comparing the bracket types. The use of DIAGNOdent has some limitations, but may prove to be useful to monitor white spot lesions longitudinally.* ORTHODONTICS (CHIC) 2011;12:XXX–XXX.

Key words: *microbial colonization, white spot lesions, elastomeric-ligated appliances, self-ligating appliances*

The development of the acid-etch bonding technique has revolutionized the placement of fixed orthodontic appliances. Although the bonding of brackets provides many benefits, formation of white spot lesions is a common adverse effect. Numerous studies have shown that there is an increase in caries-causing bacteria when fixed orthodontic appliances are placed.[1–8] Due to increased difficulty in cleaning once orthodontic appliances are placed, there is an increase in plaque accumulation and development of white spot lesions.[4,7–12] For example, Gorelick et al[9] found an increase in the prevalence of at least one white spot lesion in 50% of treated patients, compared to 24% of untreated subjects.

Bacteria considered most significantly involved with caries lesions include *Streptococcus mutans*, *Actinomyces*, and *Lactobacillus* species. These cariogenic bacteria can cause enamel demineralization (white spot lesions and subsequent cavitation) via the accumulation of acid by-products, most notably lactic acid, as a result of the metabolism of simple carbohydrates.[1,2,7,8,13] Placement of metal brackets has been found to enhance the presence of *S mutans*, which is considered among the first to colonize brackets.[1,2]

Rapid adenosine triphosphate (ATP)–driven bioluminescence assays have long been used as a quantitative measure of microbial numbers, more recently in dental plaque, including plaque from patients in the authors' original orthodontic study.[14,15] Bioluminescence assays measuring energy molecules, including ATP, have demonstrated statistical correlations with plaque mass obtained from both humans and animals.[14,15]

There are many variations among fixed orthodontic appliances used today, but ligation method divides them into two major categories: conventional ligation (using elastomeric modules or wire ligation) and self-ligation (ligation mechanism in bracket). Studies have evaluated the microbial colonization of conventional brackets associated with ligation wires vs elastomeric modules, and while some found no significant difference in plaque accumulation,[8,12] others found increased plaque accumulation with the use of elastomeric-ligated appliances.[4,14] Recently, our group[14] quantified plaque bacteria around self-ligating vs conventional elastic-ligated brackets and found that at 5 weeks postbonding, there was significantly lower plaque accumulation around self-ligating brackets. The vendors for self-ligating brackets and some orthodontists claim reductions in the amount of plaque accumulation surrounding self-ligating brackets, but further studies are required to substantiate such claims.[16]

With reduction in accumulation of plaque, suggestions have been made that self-ligating brackets will also promote reductions in the development of white spot lesions. Findings by our group published in Pellegrini et al[14] provide suggestive evidence that reduction in white spot lesions may be a possible benefit of self-ligating brackets, although further long-term study was deemed necessary.

Methods for documenting demineralization and white spot lesion formation include digital photography and laser-light fluorescence. Digital photographs taken from multiple angles are an inexpensive and convenient method of evaluating the changes in the appearance of tooth enamel over time. In addition, images can be archived and subsequently evaluated in random sequence, allowing for assessor blinding and an unbiased detection of developing white spot lesions.[17,18] Although useful for evaluating advanced demineralization, significant mineral loss in enamel is required before white spot lesions are readily visible to the eye.[11] Early mineral loss can be detected by use of laser-light fluorescence, such as the DIAGNOdent (Kaltenbach & Voigt; distributed by KaVo America), a portable red laser-light fluorescent detector. Several studies have used the DIAGNOdent to perform in vitro studies of white spot lesions with brackets, but few have used this detection method in subjects undergoing fixed orthodontic treatment.[10,11,13,19]

"With reduction in accumulation of plaque, suggestions have been made that self-ligating brackets will also promote reductions in the development of white spot lesions."

Using the same patient sample set described in Pellegrini et al,[14] we have now completed a 1-year follow-up to the original pilot study. The purposes of this follow-up study were *(1)* to further assess the use of ATP-driven bioluminescence as an accurate measure of total plaque bacteria in orthodontic patients, *(2)* to measure and compare the levels of total plaque bacteria on the tooth surface at the periphery of the bracket-tooth interface associated with elastomeric-ligated and self-ligating orthodontic brackets after 1 year of treatment, and *(3)* to analyze white spot lesion formation on surfaces surrounding both elastomeric-ligated and self-ligating brackets, as determined by visual inspection and laser light fluorescence (DIAGNOdent).

METHODS

Patient demographics and appliance placement

Thirteen of the 14 subjects who completed the study by Pellegrini et al[14] were enrolled in this follow-up study. The original criteria for selection of subjects in the Pellegrini et al[14] study were 12 years of age or older at the start of treatment (mean, 13.9; range, 12.1 to 17.2 years) and demonstration of good oral health. All patients originally selected were diagnosed as needing fixed appliance orthodontic treatment and were not missing any lateral incisors. Patients were excluded if pregnant, diabetic, or using mouthrinses or interacting medications, including antibiotic therapy within 3 months prior to the study. One subject (patient 13) who finished the Pellegrini et al[14] study dropped out of the longitudinal study at the time of the 1-year follow-up appointment. Patient 13 (male, 11.7 years of age) was relatively unique because of his left-handedness (one of only three left-handed individuals in the study) and received only maxillary appliances (one of only two individuals in the study) instead of full orthodontic treatment. At the time of bracket placement, none of the patients had visual white spot lesions on their lateral incisors. All patients were treated at the Oregon Health & Science University (OHSU) Department of Orthodontics, and the OHSU Institutional Review Board approved the human subjects' protocol prior to the initiation of the study.

The two different brackets were bonded to lateral incisors by random allocation using a split-mouth design (Fig 1a). Initially, in each arch, one lateral incisor received either an experimental bracket (self-ligating, 0.022-inch In-Ovation-R,

GAC) (Fig 1b) or a control bracket (standard, elastomeric-ligated, 0.022-inch Mini-Ovation, GAC) (Fig 1c) while the contralateral incisor received the other type of bracket. The right-left distribution of elastomeric-ligated vs self-ligating brackets was evenly distributed among patients. Lesaffre et al[20] discussed the methodologic aspects of the split-mouth design with regard to selected CONSORT guidelines for cluster-randomized clinical trials. The split-mouth design was important because right- and left-handed individuals tend to spend more time brushing on the contralateral side.[21,22] There were four males and nine females. Ten subjects were right-handed, two were left-handed, and one was ambidextrous. The complete patient population profiles as described above, including information concerning bracket-tooth assignments, can be found in Table 1 of the Pellegrini et al[14] study. The appliances were bonded using composite resin (Neo-bond, GAC), with all but the assigned lateral incisors bonded with 0.022-inch self-ligating brackets (In-Ovation-R). Oral hygiene instructions were presented and included emphasis on brushing after each meal, use of proxy brushes and floss threaders once a day, and the option of using mouthrinses to supplement these measures.

Plaque was collected from all four lateral incisors with the exception of one patient (patient 5), where only the maxillary lateral incisors were included (this patient had only maxillary appliances). At the 1-year sampling visits, the elastomeric modules were removed or the self-ligating mechanism disengaged and the archwires removed. Plaque specimens were obtained from around the bracket base of each lateral incisor utilizing a sterilized dental scaler (no. 8/9 Orban DE hoe scaler, Hu-Friedy). A four-pass technique[14] was employed where the investigator moved the instrument tip around the circumference of the bracket. After plaque collection, stimulated saliva specimens were also collected from each patient.

Microbiologic analysis of samples

Four plaque samples (one from each lateral incisor, with the exception of patient 5) were collected per subject 1 year after treatment was started. Each plaque sample was diluted in 1 mL of sterile phosphate-buffered saline (PBS), glass beads were added, and the samples were dispersed by vigorous agitation on a rocker platform (37°C, 10 minutes). The dispersed plaque samples, in addition to stimulated saliva specimens, then underwent 10-fold serial dilutions in PBS. The dilutions were plated on enriched blood agar (PML Microbiologicals) to determine total bacterial numbers.

ATP-driven bioluminescence of samples

ATP levels contained in bacteria from diluted plaque and saliva were assessed with the BacTiter Glo Microbial Cell Viability Assay kit (Promega; product no. G8231), which contains luciferin substrate and luciferase enzyme required to drive the conversion of ATP to ADP, resulting in the generation of measureable light.[15] ATP-driven bioluminescence was measured using a Veritas Microplate luminometer (Turner Biosystems). Relative light units (RLUs) were calibrated using a standard curve of ATP (pM concentrations or greater; powdered chemical obtained from Sigma Chemical) and correlated against optical density (absorbance at 600 nM wavelength measured with Novaspec II Visible spectrophotometer). A 10^5-fold dynamic range in RLU readouts was obtained using this method with the Veritas luminometer.

Photographs and laser-fluorescence procedures

Immediately after the bonding appointment and immediately after the 1-year plaque collection, the lateral incisors were thoroughly cleaned and briefly air dried. Photographs were taken using a standardized technique where a jig

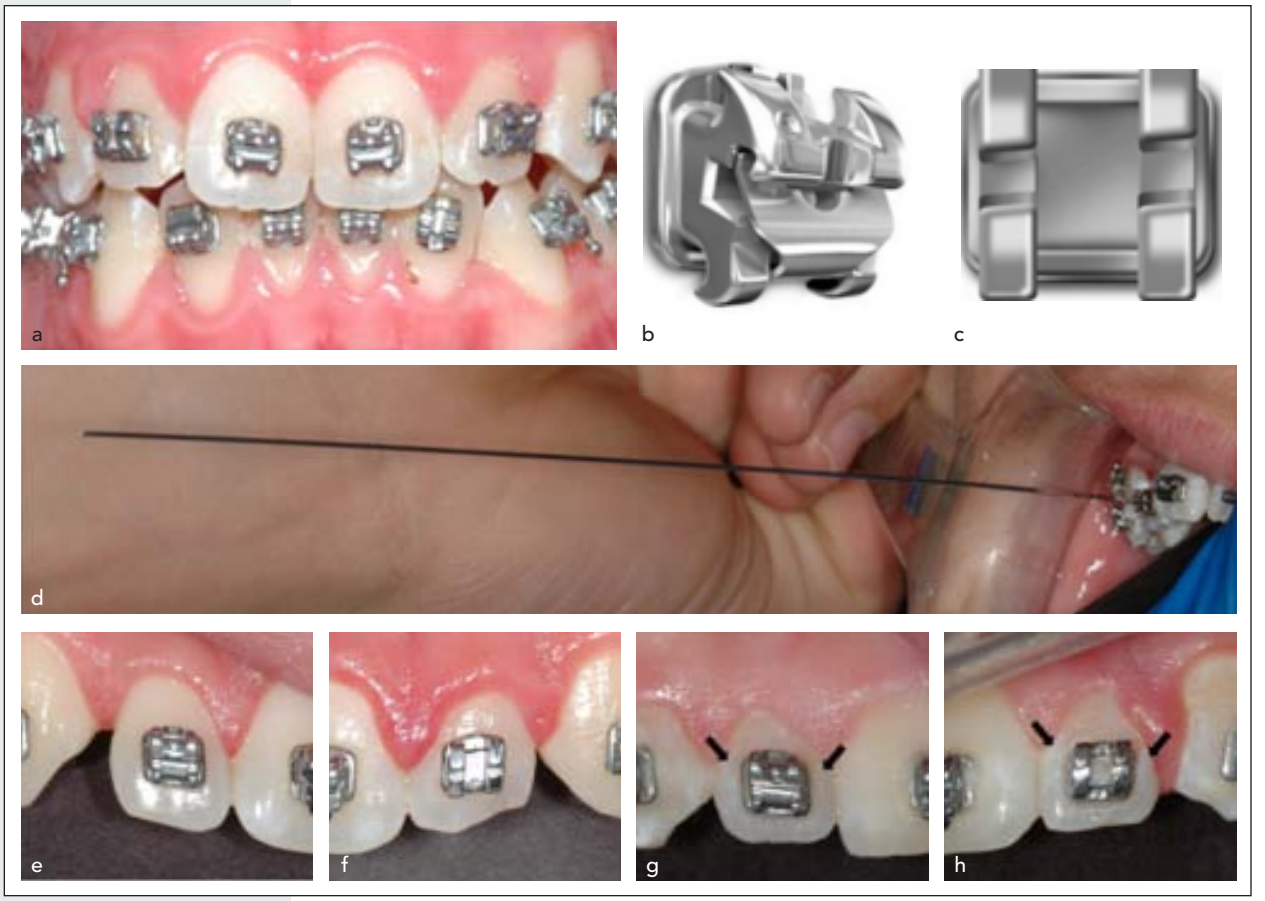

Fig 1 *(a)* Photograph of a subject with self-ligating and elastomeric-ligated brackets bonded on the lateral incisors. All remaining teeth were bonded with self-ligating brackets. *(b)* In-Ovation-R self-ligating bracket. *(c)* Mini-Ovation elastomeric-ligated bracket. *(d)* 15-cm long 0.021 × 0.028-inch stainless steel jig tied into the bracket on a lateral incisor to standardize the focal distance on the digital camera. *(e and f)* Standardized photographs showing no presence of white spot lesions. *(g and h)* Standardized photographs showing visual white spot lesions (arrows).

(Fig 1d) was used to establish a 15-cm distance from the buccal surface of a tooth to the camera lens.[17] Three images of the lateral incisors were made, one perpendicular to the buccal surface and two at approximately 20 degrees above and 20 degrees below perpendicular to the buccal surface, as assessed by the eye.[18,23–25] The three images were coded and archived for white spot lesion evaluation. Evaluation for the presence of white spot lesions was conducted with the images viewed at random and scored using a modified Gorelick index,[9] according to the following criteria: no white spot formation (0), slight white spot formation (1), severe white spot formation (2), and white spot formation with cavitation (3). The scores based on the photographs (for example, Figs 1e to 1h) were used to correlate visual white spot lesion formation to laser-light fluorescence readings. For an assessment of reliability, the photographs were blindly re-evaluated weeks later, and the scoring results were compared.

Table 1 Composite from the present study and Pellegrini et al[14] of total bacterial numbers and ATP-driven bioluminescence units on teeth ligated with self-ligating and elastomeric-ligated brackets

	Total bacterial no.				ATP-driven bioluminescence units			
	Self-ligating		Elastomeric-ligated		Self-ligating		Elastomeric-ligated	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 wk postbonding†	2.00E + 6	2.46E + 6	5.00E + 6	7.59E + 6	3.56E + 7	3.65E + 7	7.80E + 7	9.02E + 7
5 wks postbonding†	2.00E + 6	4.23E + 6	3.00E + 6	4.68E + 6	8.90E + 7	1.21E + 8	1.18E + 8	1.31E + 8
1 y postbonding	1.52E + 7	2.11E + 7	1.13E + 7	1.66E + 7	2.20E + 8	4.09E + 8	2.21E + 8	3.61E + 8

SD, standard deviation.
†Time points containing data collected in the Pellegrini et al[14] study.

For laser-light fluorescence readings (DIAGNOdent), a technique similar to that of Staudt et al[11] was used. The sample teeth were isolated with cotton rolls and air-dried for a few seconds; measurements were obtained from tooth surfaces along the four sides of the bracket (gingival, incisal, mesial, and distal). The tapered tip (tip A) was used at approximately 60 degrees to the tooth surface to allow measurement of the enamel closest to the bracket.[11] The largest unit (maximum range, 0 to 99) was recorded for each of the four sides. Based on the manufacturer's recommendations, we assessed that readings < 5 represent no lesions and values ≥ 5 to 99 represent increasing severity of lesions. For evaluation of method error, a second set of readings was made on four subjects selected at random. DIAGNOdent method error was calculated using the Dalberg formula: $s=\sqrt{\Sigma d^2/2n}$, in which d is the difference between the two measurements at a site and n is the number of sites measured.

Statistical analysis

Descriptive statistics, including measures of central tendency, variability, distribution characteristics, and Pearson correlations, were calculated. The distributions for total bacterial numbers and ATP-driven bioluminescence determinations (in RLUs) were severely positively skewed; thus, a natural logarithmic transformation of these variables was applied to normalize the distributions. However, an examination of the distributions revealed two observations to be greater than two standard deviations from the mean, and these data points were winsorized to reduce the influence of these extreme outliers on the analysis. All statistical analyses were performed on the transformed variables. The variables were then back-transformed to return the results to the original measurement scale for presentation of means and confidence intervals.

Mixed-design full factorial analyses of variance (ANOVAs) ($2 \times 2 \times 2$) were conducted to determine whether there were any significant patterns of differences in total plaque bacteria counts and ATP-driven bioluminescence values between elastomeric-ligated and self-ligating brackets. The split-mouth design can be considered an adaptation of the split plot design that has its roots in

agricultural research.[20] Our statistical analysis included both within-subject variables, such as bracket assignment (elastomeric-ligated vs self-ligated) and dental arch (maxillary vs mandibular), where the effects on teeth within individuals were correlated, and between-subject variables, which in our study consisted of the pattern of bracket assignment. The pattern of bracket assignment (elastomeric-ligated/self-ligated/elastomeric-ligated/self-ligated vs self-ligated/elastomeric-ligated/self-ligated/elastomeric-ligated) corresponds to the following teeth: maxillary right lateral incisor (site 1), maxillary left lateral incisor (site 2), mandibular left lateral incisor (site 3), and mandibular right lateral incisor (site 4). Based on the Cohen effect size guidelines,[26] a factor was considered to have a significant effect on the overall variance if an omega squared (ω^2) value was < .06.[26]

To explore the correspondence of DIAGNOdent white spot lesion measurements with photographic-visual white spot lesion indices (using the latter as the gold standard), we calculated the sensitivity (number of correct DIAGNOdent positive white spot lesion identifications/[number of correct DIAGNOdent positive white spot lesion identifications + number of incorrect DIAGNOdent negative white spot lesion identifications]) and specificity (number of correct DIAGNOdent negative white spot lesion identifications/[number of correct DIAGNOdent negative white spot lesion identifications + number of incorrect DIAGNOdent positive white spot lesion identifications]).

RESULTS

Statistical correlation linking ATP-driven bioluminescence values to total plaque bacterial number

Using plaque specimens (n = 50) collected from all patients, serial dilution plating of each specimen was conducted for quantification of total plaque bacteria using enriched medium (blood agar). When ATP-driven bioluminescence values were determined and compared to bacterial cell number, a correlation of 0.80 was determined for total plaque bacteria. When these ATP-driven bioluminescence readings were analyzed using plaque (n = 50) and saliva specimens (n = 13) obtained from each patient, thus increasing the total n value to 63 specimens, a correlation of 0.90 was determined for total oral bacteria. Thus, ATP-driven bioluminescence is highly predictive of numbers of total plaque and total oral bacteria in orthodontic patients.

Findings of plaque bacterial load and orthodontic brackets

The mean bacterial numbers (based on plating using enriched medium) contained in plaque surrounding the elastomeric-ligated and self-ligating brackets are shown in Table 1. The mean bacterial numbers for each appliance, comparing the 1-week and 5-week postbonding numbers determined by Pellegrini et al[14] vs the 1-year postbonding numbers determined in this current study, were higher for the 1-year collection. The results from the full-factorial ANOVA showed omega squared (ω^2) values ranging from .04 to < .001, indicating there was little to no effect related to bracket type, dental arch, or pattern of bracket assignment (Table 2). For bacterial numbers, the estimated marginal means and associated confidence intervals showed similar values between bracket type, dental arch, and dental arch by bracket type (Table 3). Thus, these results did not demonstrate significant differences in the numbers of total plaque bacteria surrounding the two different bracket types (elastomeric-ligated vs self-ligating) or between dental arches (maxillary vs mandibular) or pattern of bracket assignment (elastomeric-ligated/self-ligating/elastomeric-ligated/self-ligating vs self-ligating/elastomeric-ligated/self-ligating/elastomeric-ligated) after 1 year of orthodontic treatment.

Table 2 ANOVA for log of total bacterial numbers and ATP bioluminescence for specimens collected 1 year postbonding

	Total bacterial no.			ATP bioluminescence units		
Source	F[a]	*P*	Ω^2	F[b]	*P*	Ω^2
Pattern of bracket assignment (EL/SL/EL/SL vs SL/EL/SL/EL)	.78	.40	< .01	.22	.65	< .01
Arch (maxillary vs mandibular)*	.01	.92	< .01	4.62	.06	.07
Bracket type (elastomeric-ligated vs self-ligating)	.35	.57	< .01	.18	.68	< .01
Arch by pattern of bracket assignment	2.08	.18	.01	.47	.51	< .01
Arch by bracket type	.02	.89	< .01	.05	.83	< .01
Bracket type by pattern of bracket assignment	3.14	.11	.04	.91	.37	< .01
Arch by bracket type by pattern of bracket assignment	1.12	.32	.01	1.45	.26	.02

a= df is (1,10) for each F test, b = df is (1,9) for each F test, F = F ratio, *P* = Type I error probability of given F ratio, ω^2 = unbiased estimate of the proportion of variance accounted for in dependent variable by a factor EL, elastomeric-ligated; SL, self-ligating.
ATP bioluminescence units are in RLUs or relative light units.
*Dental arch independent of bracket type or pattern of bracket assignment appear to account for variance in ATP-driven bioluminescence values (ω^2 = 0.073, a medium effect).

> *". . . little or no differences were observed between the elastomeric-ligated and self-ligating brackets after 1 year of orthodontic treatment."*

Findings of ATP-driven bioluminescence and orthodontic brackets

The mean ATP-driven bioluminescence values for plaque surrounding the elastomeric-ligated and self-ligating brackets are shown in Table 1. Similar to the mean bacterial numbers data, the mean ATP-driven bioluminescence values observed for the 1-year collection were higher than corresponding values obtained at the 1-week and 5-week postbonding collections. The results from the full factorial ANOVA demonstrate no significant differences in ATP-driven bioluminescence based on bracket type (elastomeric-ligated vs self-ligating) or pattern of bracket assignment (elastomeric-ligated/self-ligating/elastomeric-ligated/self-ligating vs self-ligating/elastomeric-ligated/self-ligating/elastomeric-ligated) after 1 year of orthodontic treatment (Table 3). Of the factors tested in this analysis, only dental arch independent of bracket type or pattern of bracket assignment appeared to account for variance in ATP-driven bioluminescence values (ω^2 = 0.073, a medium effect[26]). The estimated marginal means and associated confidence intervals for the ATP-driven bioluminescence data for each dental arch and difference in arch values are presented in Table 3. The mean ATP-driven bioluminescence value in the maxillary arch (GM [or geometric mean] = 8.160E + 7) was less than in the mandibular arch (GM = 1.315E + 8), with a mean difference of 4.995E + 7 (95% confidence interval: –2.034E + 6 to 1.359E + 8). When specifically examining the effect of the elastomeric-ligated vs self-ligating brackets on ATP-driven bioluminescence, these data support the bacterial numbers data, where little or no differences were observed between the elastomeric-ligated and self-ligating brackets after 1 year of orthodontic treatment.

Table 3 Back transformed (geometric means) total bacterial numbers and ATP bioluminescence units on teeth ligated with self-ligating and elastomeric-ligated brackets for specimens collected at 1-year postbonding*

		Total bacterial no.			ATP bioluminescence units (RLUs)		
			95% confidence interval			95% confidence interval	
		Mean	Lower bound	Upper bound	Mean†	Lower bound	Upper bound
Bracket type	Self-ligating	3.91E + 6	1.40E + 6	1.09E + 7	9.94E + 7	5.73E + 7	1.73E + 8
	Elastomeric-ligated	4.65E + 6	1.80E + 6	1.20E + 7	1.08E + 8	5.37E + 7	2.17E + 8
Arch	Mandibular	4.30E + 6	1.67E + 6	1.11E + 7	1.32E + 8	7.87E + 7	2.20E + 8
	Maxillary	4.22E + 6	1.61E + 6	1.11E + 7	8.16E + 7	3.87E + 7	1.72E + 8
Arch by bracket type	Mandibular SL	3.81E + 6	1.20E + 6	1.21E + 7	1.21E + 8	6.02E + 7	2.44E + 8
	Mandibular EL	4.86E + 6	2.09E + 6	1.13E + 7	1.43E + 8	9.32E + 7	2.18E + 8
	Maxillary SL	4.01E + 6	1.12E + 6	1.44E + 7	8.15E + 7	3.54E + 7	1.88E + 8
	Maxillary EL	4.45E + 6	1.27E + 6	1.56E + 7	8.17E + 7	2.84E + 7	2.35E + 8

*The distributions for total bacterial numbers and ATP-driven bioluminescence (in RLUs) were positively skewed; thus, a natural logarithmic transformation of these variables were applied to normalize the distributions. However, an examination of the distributions revealed two observations to be greater than two standard deviations from the mean, and these data points were winsorized to reduce the influence of these extreme outliers on the analysis. All statistical analyses were performed on the transformed variables. The variables were then back-transformed to return the results to the original measurement scale for presentation of means (geometric means) and confidence intervals.
†The comparison of the mean ATP-driven bioluminescence values for the mandibular and maxillary arches have an ANOVA where $\omega^2 = 0.073$, a medium effect.

White spot lesion findings

The modified Gorelick index[9] scores for photographic evaluation showed slight white spot lesions were present on seven of the 50 (14%) lateral incisors, specifically occurring in patients 3, 16, and 18 (Table 4). Three white spot lesions, as determined by the modified Gorelick index scores, were associated with elastomeric-ligated brackets and four white spot lesions were associated with self-ligating brackets. Repeat, blinded white spot lesion assessment from the photographs yielded agreement in scoring with all teeth. For DIAGNOdent readings, 11 of the 50 (22%) lateral incisors had readings ≥ 5, indicating presence of white spot lesions (Table 4). Eight teeth had readings of 5 to 10, and three had readings ≥ 11. Six white spot lesions, as determined by DIAGNOdent, were associated with elastomeric-ligating brackets and five white spot lesions were associated with self-ligating brackets (Table 4). DIAGNOdent method error was determined to be 0.29 units. Thus, using the photographic evaluation as the gold standard, DIAGNOdent identified five teeth that scored a visual white spot lesion (true positives), whereas there were six teeth with no visual white spot lesions that had DIAGNOdent measurements ≥ 5 (false positives). Two teeth with slight white spot lesions by photographic evaluation had DIAGNOdent measurements < 5 (false negatives). When compared to the modified Gorelick white spot lesion index scores, the sensitivity and specificity of the DIAGNOdent measurements were computed to be 0.71 and 0.88, respectively.

Table 4 Comparison of photographic-visual white spot lesion indices with DIAGNOdent white spot lesion measurement

	Site 1		Site 2		Site 3		Site 4	
Patient	DIAGNOdent reading	Visual white spot lesion	DIAGNOdent reading	Visual white spot lesion	DIAGNOdent reading	Visual white spot lesion	DIAGNOdent reading	Visual white spot lesion
1	4	0	3	0	2	0	3	0
2	3	0	3	0	2	0	1	0
3	6 (SL)†	1 (SL)†	7 (EL)†	1 (EL)†	3	0	14 (EL)*	0
4	3	0	1	0	1	0	2	0
5	3	0	3	0				
6	1	0	1	0	1	0	1	0
7	1	0	1	0	1	0	2	0
8	2	0	2	0	4	0	16 (SL)*	0
12	6 (EL)*	0	2	0	2	0	3	0
14	3	0	3	0	5 (SL)*	0	6 (EL)*	0
16	8 (SL)†	1 (SL)†	1	0	1	0	6 (EL)*	0
17	2	0	3	0	1	0	2	0
18	7 (SL)†	1 (SL)†	26 (EL)†	1 (EL)†	2	1 (SL)*	2	1 (EL)*

DIAGNOdent measurements > 5 are considered a white spot lesion.
Modified Gorelick Index scores: 0, no white spot lesion; 1, slight white spot lesion; 2, severe white spot lesion; 3, cavitation. The highest values for any given site were recorded (DIAGNOdent measurement and index scores).
*indicates where findings of white spot lesions differed using DIAGNOdent and visual methods
†indicates where findings of white spot lesions were similar using DIAGNOdent and visual methods.
Site 1, maxillary right lateral incisor; site 2;maxillary left lateral incisor; site 3, mandibular left lateral incisor; site 4, mandibular right lateral incisor.
SL or EL are assigned in parentheses for DIAGNOdent measurements > 5 or modifed Gorelick Index > 1.

DISCUSSION

ATP-driven bioluminescence was used in this study as a simple rapid tool for the quantification of total oral bacteria. As with the results of Pellegrini et al,[14] where ATP-driven bioluminescence values correlated well with total oral bacterial numbers ($r = 0.90$), in this follow-up study, ATP-driven bioluminescence was also found to correlate strongly with total oral bacterial numbers 1 year postbonding ($r = 0.90$). It can be concluded that ATP-driven bioluminescence is highly predictive of total oral bacterial load. Thus, ATP-driven bioluminescence assays could be used as a rapid chairside clinical test to monitor total plaque bacteria in patients. This information could be used to quantitatively evaluate the effectiveness of oral hygiene in patients and to potentially determine efficacy of intervention therapies aimed at minimizing white spot lesions.[14,15]

Two brackets types were utilized to assess the accumulation of plaque bacteria associated with different ligation mechanisms in this 1-year follow-up of our original short-term pilot study. Of the 14 subjects who completed the original Pellegrini et al[14] study, 13 were available for the 1-year specimen collection. In the Pellegrini et al[14] study, higher total bacterial numbers were obtained surrounding the elastomeric-ligated brackets than the self-ligating brackets at 1 week postbonding and 5 weeks postbonding (Table 1). Forsberg et al[4] found similar results showing that ligation with elastomeric rings was associated with higher mean

numbers of bacteria at every collection time point compared to ligation with stainless steel wires. The Forsberg et al[4] study included 12 subjects in whom one side of the midline brackets were ligated with elastomeric rings and the other side with steel ligatures, and plaque samples were collected at 4, 10, 19, 34, and 61 weeks.

Contrary to our earlier findings in the Pellegrini et al[14] study and the implications of the Forsberg et al[4] study, it was determined in the current pilot study that with different orthodontic brackets, the difference in plaque bacterial load found early in orthodontic treatment disappears after 1 year of orthodontic treatment. The disappearance of differences in total plaque bacteria surrounding the two bracket types after 1 year of orthodontic treatment may be due to decreases in patient compliance with oral hygiene practices. It is generally recognized that through the course of treatment, patients often become less compliant, including their oral hygiene practices. It is unknown if this lack of difference can be generalized to all types of self-ligating brackets or if design differences among self-ligating brackets could result in variations in plaque formation (eg, due to differences in the extent of plaque retained within the brackets). It is interesting that there is a potential difference in ATP-driven bioluminescence values observed between the maxillary and mandibular arches independent of bracket type or pattern of bracket assignment (Tables 2 and 3). We recognize that our sample size in patient numbers was small, and future investigations would benefit from a larger sample size.

The observations of Türkkahraman et al[8] are consistent with this study in that no significant differences in plaque surrounding brackets ligated by two different methods were found. Their observations included 21 subjects undergoing orthodontic treatment, in which the maxillary brackets on the right side were ligated with elastomeric rings and those on the left side were ligated with stainless steel wire ligatures, and microbial samples were collected prior to bonding, 1 week after bonding, and at 5 weeks.[8] Türkkahraman et al[8] found that although microbial counts were slightly higher in the elastomeric group, there was no significant difference between groups. In a recent study using 32 subjects, Pandis et al[27] found that the levels of *S mutans* in saliva did not significantly differ between patients using conventional vs self-ligating brackets.

In the present pilot study, the DIAGNOdent was found to accurately identify five of seven photographic white spot lesions, indicating a moderate sensitivity of 0.71, and was found to more accurately identify absence of photographic white spot lesions with a specificity of 0.88 (Table 4). Of the 50 teeth involved in our study, only 3 teeth (6%; in patients 3, 8, and 18) had DIAGNOdent measurements > 10 (Table 4). In a study by Gorelick et al[9] where orthodontic patients were evaluated visually for white spot lesions, the prevalence of white spot lesions was 10.8% in the 2,211 teeth examined. They found the prevalence of white spot lesions among maxillary lateral incisors to be 23%,[9] compared to the current findings of 14% using the visual-photographic evaluation (Gorelick et al[9] scores of ≥ 1) and 22% using DIAGNOdent (readings ≥ 5). Although our DIAGNOdent data compare closely to that of Gorelick et al[9] for lateral incisors, our evaluation of photographs did not agree. This lack of correspondence between white spot lesions identified by the visual-photographic method may relate to differences in sensitivity of direct visual (Gorelick et al[9]) vs the photographic method used in the current pilot study, differences in length of treatment before the white spot lesion analysis was conducted (shorter in the current study), or to differences in the study populations.

Using the photographic evaluation method, only seven teeth demonstrated white spot lesion formation, and these were about equally distributed between teeth with the two bracket types. Because the number of teeth that developed white spot lesions was relatively low, and thus our ability to test for detection of white spot lesions was limited, findings of other investigators who have utilized laser-light fluorescence for detecting white spot lesions are of interest. In

an in vitro study,[17] DIAGNOdent measurements were made around orthodontic brackets bonded to extracted third molars. Teeth selected in that study were required to have visible decalcification, and the sites selected for bonding brackets on each tooth were measured with the DIAGNOdent before and after bonding.[17] The investigators found the lesions showed a slight decrease of 0.5 units after bonding brackets, leading them to conclude that demineralization around brackets may be reliably measured by laser fluorescence in vitro.[17] Other in vitro studies have found the sensitivity and specificity of the DIAGNOdent to be reasonably good, with a range from 0.72 to 0.79 and 0.73 to 0.87, respectively.[13,19] In the present study, we found that DIAGNOdent had moderate sensitivity (0.71) and good specificity (0.88). Similar to our findings, Kronenberg et al[28] recently evaluated the development of white spot lesions in vivo around brackets during orthodontic treatment, comparing visual evaluation to DIAGNOdent readings. They found that compared to clinical evaluation, DIAGNOdent measurements were less reliable for detecting changes and concluded that visual evaluation of initial caries lesions was superior to DIAGNOdent measurements during multibracket appliance therapy.[29]

CONCLUSION

From this study, the following three conclusions were drawn:

1. ATP-driven bioluminescence values correlated to total plaque bacterial numbers (r = 0.80) and total oral bacterial numbers (r = 0.90). ATP-driven bioluminescence may serve as a simple rapid tool for quantification of plaque or oral bacterial load that can be used chairside during orthodontic treatment.
2. After 1 year of orthodontic treatment, no differences in retention of plaque bacteria were found between self-ligating and elastomeric-ligated brackets.
3. Based on photographic and DIAGNOdent determinations, white spot lesions were found at nearly equal frequency on teeth bonded with either bracket type. DIAGNOdent measurements were found to have moderate sensitivity (0.71) and good specificity (0.88) when compared to white spot lesions determined using photographic evaluation. DIAGNOdent has the potential to serve as a rapid tool for chairside monitoring of white spot lesion formation and patient education.

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