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Plaque retention by self-ligating vs elastomeric orthodontic brackets: Quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence

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Introduction: Enamel decalcification is a common problem in orthodontics. The objectives of this randomized clinical study were to enumerate and compare plaque bacteria surrounding 2 bracket types, self-ligating (SL) vs elastomeric ligating (E), and to determine whether adenosine triphosphate (ATP)-driven bioluminescence could be used for rapid assessment of bacterial load in plaque. **Methods:** Patients (ages, 11-17 years) were bonded with SL and E brackets in 14 maxillary and 12 mandibular arches by using a split-mouth design. Recall visits were at 1 week and 5 weeks after bonding. Plaque specimens were assayed for oral bacteria and subjected to ATP-driven bioluminescence determinations with a luciferin-based assay. **Results:** In most patients, teeth bonded with SL attachments had fewer bacteria in plaque than did teeth bonded with E brackets. At 1 and 5 weeks after bonding, the means for SL vs E brackets were statistically lower for total bacteria and oral streptococci ($P < 0.05$). ATP bioluminescence values were statistically correlated to the total oral bacteria and oral streptococci, with correlation coefficients of 0.895 and 0.843, respectively. **Conclusions:** SL appliances promote reduced retention of oral bacteria, and ATP bioluminescence might be a useful tool in the rapid quantification of bacterial load and the assessment of oral hygiene during orthodontic treatment. (Am J Orthod Dentofacial Orthop 2009;135:426.e1–426.e9)

The presence of acid-producing bacteria, colonizing the tooth surface and surrounding orthodontic appliances, leads to enamel demineralization and often causes alterations in the appearance of the enamel surface.[1-5] These changes are an esthetic problem that can persist for many years after treatment.[6] In addition, decalcification related to bonded orthodontic appliances appears to occur primarily near the appliance and not farther away along the facial surface.[2,7] Thus, the prevention of demineralization at the periphery of the brackets is a significant challenge to orthodontic professionals.

In recent years, the development of the acid-etch bonding technique has changed the practice of orthodontics. Before the introduction of the bonding technique, orthodontic brackets were attached to metal bands that were individually fitted and cemented to each tooth. Bonded brackets have many advantages over bands because of better esthetics, ease of placement and removal, and accessibility for oral hygiene.[8,9] Nonetheless, bonded orthodontic brackets impede good oral hygiene, resulting in plaque accumulation and significantly increased risks for enamel decalcification.

After the bonding of orthodontic appliances, there are documented increases in the amounts of cariogenic microorganisms, *Streptococcus mutans* and lactobacilli, in saliva and dental plaque of patients.[10-13] Although

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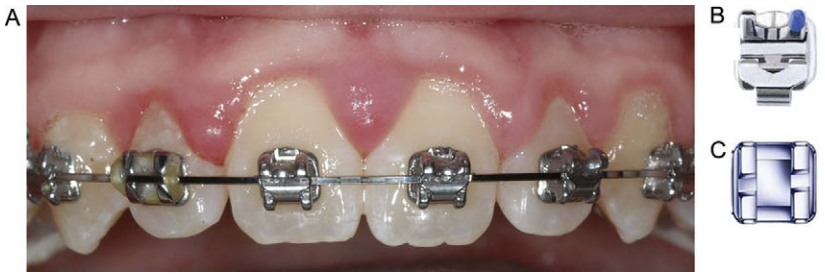

Fig 1. Experimental design and description of SL and E appliances: **A,** experimental design on a maxillary arch; **B,** SL bracket, Innovation-R; **C,** traditional E bracket, Mini-Ovation.

several studies have investigated the effects of fixed orthodontic appliances on the microbial flora profile, few studies have compared the effects of bracket architecture—specifically, the archwire ligation method—or have obtained a quantitative evaluation of the bacterial accumulation that occurs with the bonding of fixed appliances.[10,14,15]

Rapid adenosine triphosphate (ATP)-driven bioluminescence assays have long been used as a quantitative measure of microbial numbers and more recently in dental plaque.[16-19] Bioluminescence assays measuring energy metabolites, including ATP, have been shown to have high correlations with plaque mass obtained from both human and animal subjects.[16-20]

In this randomized clinical study, we compared the numbers of oral bacteria in plaque surrounding 2 distinct orthodontic brackets, self-ligating (SL) vs elastomeric ligating (E), using a split-mouth design. An additional purpose of this study was to demonstrate the use of ATP-driven bioluminescence as an innovative tool for rapid chair-side enumeration of total oral bacteria. Using plaque and saliva specimens from 14 participants, we compared ATP-driven bioluminescence from oral specimens to bacterial numbers quantified with standard microbiologic plating methods. This is the first orthodontic study to compare the hygienic effects of self-ligation vs traditional ligation methods, and demonstrates ATP-driven bioluminescence in the quantitative evaluation of bacteria around fixed appliances.

MATERIAL AND METHODS

The test subjects were 14 patients (12 with full appliances, 2 with appliances on the maxillary arch only) who successfully completed the study of the originally enrolled 18 patients; 4 patients were excluded because of failure to adhere to the selection criteria or failure to keep at least 1 appointment for specimen collection. The criteria for inclusion in this study were age (dental age, 12 years or older; mean, 13.9 years; range, 11.7-17.2 years) and demonstrated ability to maintain adequate oral health. All patients selected required fixed appliance orthodontic therapy and were scheduled for treatment in the Orthodontics Clinic at the Oregon Health & Science University (OHSU). Patients who were pregnant, diabetic, using mouth rinses or interacting medications, including antibiotic therapy within 3 months before the study, were excluded. Participants were assigned study identifier numbers that were accessible only to the study investigators and were kept in a notebook locked in the Department of Orthodontics.

This study was reviewed and approved by the OHSU Institutional Review Board. In addition to the consent form for routine orthodontic care currently used in the OHSU orthodontic clinic, the parent or guardian of each subject was given a second consent form specifically relating to this study. We requested the participants to also refrain from eating or drinking 1 hour before the sampling appointments. The study called for no additional treatments or procedures not normally performed during routine oral care for initial bonding or orthodontic adjustment visits.

This study had a randomized split-mouth design. The doctor assigned to plaque collection (P.P.) used a standardized collection technique and was solely responsible for the sample collection. At the initial bracket bonding appointment (T0), all teeth were polished with coarse-grade prophylaxis paste with a rubber cup and a slow-speed hand piece. The patients were given oral hygiene instructions, fluoridated toothpaste, and a toothbrush, and asked not to use other oral hygiene supplements during the study. At TO, half of each arch, either the left or the right side, was randomly assigned to receive the experimental bracket, with the opposite side as the control (Fig 1, *A*). For each arch, the left or the right lateral incisors randomly received either the experimental SL bracket (0.022-in; In-Ovation-R, GAC International, Bohemia, NY [Fig 1, *B*]) or the control E bracket (0.022-in; Mini-Ovation, GAC [Fig 1, *C*]); the latter brackets were ligated with silver-colored elastomeric ligatures (Alastiks, 3M Unitek, Monrovia, Calif).

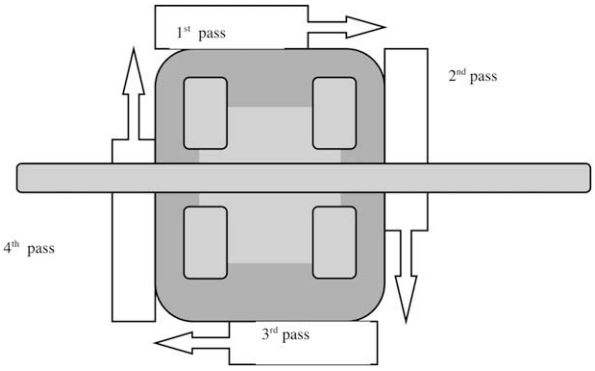

Fig 2. Four-pass sampling technique: a standardized, sterilized instrument tip is moved circumferentially around the bracket. The wire is pictured, and the ligation mechanism is carefully removed before sampling.

The appliances were directly bonded by using composite resin, with all but the lateral incisors bonded with SL brackets.

The plaque-sampling investigators used a standardized protocol to collect specimens. At each sampling visit, the operator carefully removed or disengaged the elastomeric tie or ligation mechanism and removed the archwires. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary and mandibular lateral incisors with a sterilized dental scaler with the same tip dimensions (#8/9 Orban DE hoe scaler, Hu-Friedy, Chicago, Ill). Because the area of increased decalcification was generally immediately adjacent to the brackets, a 4-pass technique was used to move the instrument tip around the circumference of the bracket at the bracket-tooth interface (Fig 2). Four passes, 1 along the tooth at the bracket interface at the gingival, mesial, distal, and occlusal aspects, were used to avoid overloading the instrument tip. All specimens from each tooth (left and right incisors of the maxillary and mandibular arches) were placed into 4 individual tubes with anonymous coding and sealed for transport to the laboratory. The coding of the specimens ensured blinding of laboratory personnel and helped to minimize experimental bias.

For the saliva collection, the participants were given a chewing-gum-shaped paraffin wax tablet and instructed to chew it for 1 to 5 minutes or until several milliliters of saliva was collected and expelled into a sterile calibrated collection container. The sample was coded with the unique participant identifier and transferred to the laboratory for evaluation. At T0, saliva was collected before placement of appliances.

Five specimens (4 plaque and 1 stimulated saliva specimens) were collected per subject at each appointment after bonding (T1 and T2, 1 week and 5 weeks after bonding, respectively), exception for T0, when only saliva specimens were collected. Each plaque specimen was diluted in 1 mL of phosphate-buffered saline solution with glass beads, and dispersed by vigorous agitation on a rocker platform (37°C, 10 minutes). The plaque samples were then subjected to 10-fold serial dilutions in phosphate-buffered saline solution and then plated on enriched blood agar (PML Microbiologicals, Wilsonville, Ore) to determine total bacterial numbers. Total oral streptococci numbers were determined by limiting dilution plating on mitis salivarius agar (Difco, Becton, Dickinson and Company, Sparks, Md), which uses high sucrose and vital dyes as selective agents. Mitis salivarius agar selects for numerous strains of oral streptococci with varying abilities to generate acid and effects on decalcification potential. All platings were conducted in quadruplicate, and plates with colony numbers between 50 and 500 were counted and averaged to determine mean values.

For the determination of ATP-driven bioluminescence, we used the luciferin substrate and luciferase enzyme, so that bacterial ATP could be quantitated by measuring the release of visible light.[16] ATP in bacteria from plaque specimens was determined with the BacTiter Glo Microbial Cell Viability Assay kit (G8231, Promega, Madison, Wis), with ATP-driven bioluminescence measured by the Veritas Microplate luminometer (Turner Biosystems, San Diego, Calif). Relative light units (RLUs) were calibrated by using a standard curve of ATP (picomolar concentrations or greater; powdered chemical from Sigma Chemical, St Louis, Mo) and correlated against optical density (absorbance at 600 nM wavelength measured with a Novaspec II Visible spectrophotometer, GE Healthcare Life Sciences, Uppsala, Sweden). The luminometer has a 5-fold dynamic range in RLU readouts.

Statistical analysis

Eighteen patients were enrolled to account for dropouts (Table I); 14 patients completed the study, with 12 in full appliances, and 2 receiving maxillary appliances only. Descriptive statistics, including means values for bacterial counts and corresponding standard deviations were calculated. The mean bacterial counts and the ATP-driven bioluminescence determinations (in RLUs) from teeth bonded with SL and E brackets were tested for significant differences by using paired t tests (1-tailed, with $P < 0.05$ considered statistically significant). Based on the results of the bacterial counts and ATP-driven bioluminescence determinations for each plaque sample from the lateral incisors, contrasting bracket pairs in each arch were assigned to 1 of the following groups: those with the highest bacterial counts or

Table I. Patient demographics and placement of brackets on selected teeth

*Patient**	*Sex*	*Age (y)*	*Handedness*	*Oral health*	*Average time (h) brushing before visits*†	*Orthodontic treatment*‡	*UR tooth 2 bracket type*§	*UL tooth 2 bracket type*§	*LL tooth 2 bracket type*§	*LR tooth 2 bracket type*§
1	M	14.6	Right	Good	1	Full	E	SL	E	SL
2	M	14.1	Right	Good	1	Full	E	SL	E	SL
3	F	16.1	Right	Good	1	Full	SL	E	SL	E
4	F	14.0	Right	Good	3	Full	SL	E	SL	E
5	F	17.2	Ambidextrous	Good	1	Maxillary	E	SL	N/A	N/A
6	F	12.2	Right	Good	1	Full	SL	E	SL	E
7	F	12.9	Right	Good	1	Full	E	SL	E	SL
8	F	15.9	Right	Good	2	Full	E	SL	E	SL
12	F	12.1	Left	Good	N/A	Full	E	SL	E	SL
13	M	11.7	Left	Good	N/A	Maxillary	E	SL	N/A	N/A
14	F	12.1	Right	Poor	3	Full	SL	E	SL	E
16	M	13.1	Right	Good	N/A	Full	SL	E	SL	E
17	F	15.7	Left	Good	N/A	Full	SL	E	SL	E
18	M	13.1	Right	Fair	N/A	Full	SL	E	SL	E

M, Male; *F,* female; *N/A,* not applicable; *UR tooth 2,* maxillary right lateral incisor; *UL tooth 2,* maxillary left lateral incisor; *LL tooth 2,* mandibular left lateral incisor; *LR tooth 2,* mandibular right lateral incisor.
*All patients live in Portland, Ore, and surrounding area; †All patients refrained from eating for at least 1 hour before visits; ‡No patient had active caries when orthodontic appliances were placed; §*E,* elastomeric-ligating bracket; *SL,* self-ligating bracket.

Table II. Total bacteria and oral streptococci on teeth with self-ligating and elastomeric brackets

	1 Week postbonding (T1)					*5 Weeks postbonding (T2)*				
	Self-ligating		*Elastomeric*			*Self-ligating*		*Elastomeric*		
Bacteria type	*Mean**	*SD**	*Mean**	*SD**	*P*	*Mean**	*SD**	*Mean**	*SD**	P
Total bacteria	2.00	2.46	5.00	7.59	0.017†	2.00	4.23	3.00	4.68	0.032†
Oral streptococci	0.70	1.17	2.00	4.02	0.044†	0.50	1.37	2.00	4.05	0.030†

*All mean and SD values should be multiplied by 10^6 to provide corrected values; †Values statistically significant at the 95% confidence level ($P < 0.05$).

RLUs on the tooth bonded with an SL bracket (SL >E) or those with the highest bacterial counts or RLUs on the tooth bonded with an E bracket (E >SL). The chi-square test and the distribution were used to establish whether there was a significant difference between the numbers of contrasts in each category.[10]

RESULTS

Table I describes the patients in this study, including the randomized allocation of brackets bonded to the lateral incisors. Seven patients had E brackets placed on the maxillary right quadrant and SL brackets on the maxillary left quadrant, and 7 had SL brackets on the maxillary left quadrant and E brackets in the maxillary right quadrant. Also, in all patients, the appliance type was switched in its left-right orientation when appliances were placed in the mandibular arch. Ten subjects were right-handed, 3 were left-handed, and 1 was ambidextrous. Plaque was collected from lateral incisors in the maxillary right, maxillary left, mandibular left, and mandibular right quadrants.

A higher bacterial plaque load was observed surrounding the E appliances. Table II illustrates the mean bacterial numbers for total bacteria and oral streptococci in plaque around the SL and E brackets at T1 and T2. In all cases described in Table II, the bacterial numbers were greater in plaque surrounding the E bracket than the SL bracket. Significant statistical comparisons were identified primarily when using data pooled for both arches. By using the combined data set, higher total bacterial numbers were obtained surrounding the E brackets vs SL brackets at both T1 ($P = 0.017$) and T2 ($P = 0.032$). Total streptococci numbers were also higher in plaque surrounding the E brackets vs the SL brackets at T1 ($P = 0.044$) and T2 ($P = 0.030$). For all data groups examined, the standard deviations for the mean values in each group were quite high, most likely reflecting the variability in oral hygiene among the patients. Even though the patients were asked not to brush immediately before visits, we believe that not all patients were compliant. This is also consistent with the variable amounts of visible

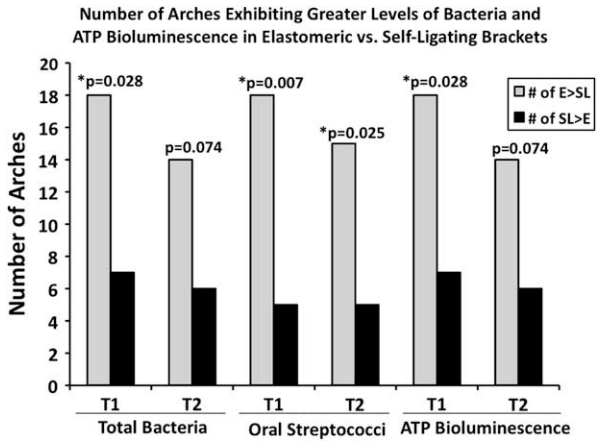

Fig 3. Intra-arch comparison of bacterial numbers and ATP bioluminescence values from plaque on tooth surfaces surrounding SL vs E appliances. Histograms depict numbers of arches in intra-arch comparisons, where plaque bacteria (either total bacteria or oral streptococci) or ATP bioluminescence values are higher on tooth surfaces surrounding E compared with SL appliances (E >SL; *gray boxes*) and the reverse comparison (SL >E; *black boxes*) for any patient. Data were analyzed by using numbers for the first and second recall visits (T1 and T2). *P* values are shown for every comparison. Statistically significant comparisons are denoted with an asterisk and are identified with *P* values <0.05.

plaque collected between patients. Thus, to account for interpatient variability, we used the split-mouth study design to also compare plaque bacteria in intra-arch bracket pairs in the patients' mouths.

Figure 3 illustrates intra-arch bracket pair comparisons divided on the basis of those with higher numbers of plaque bacteria on teeth bonded with E vs SL brackets. Histogram bar graphs are shown as numbers of contrasts where bacterial numbers, for either total bacteria or oral streptococci, surrounding the E bracket were greater than bacterial numbers surrounding the SL bracket (E >SL), as well as the reverse comparison (SL >E).

By using these intra-arch bracket pair comparisons, in most patients, at T1 and T2, teeth bonded with E brackets had higher numbers of bacteria than teeth bonded with SL brackets. Specifically, more patients (and their arches) had higher levels of plaque bacteria surrounding the E appliances compared with the SL appliances (E >SL comparison) at T1 ($P = 0.028$, Fig 3). Interestingly, when the data were split into maxillary and mandibular arches for T1 and examined to determine numbers of arches where plaque bacteria were higher on tooth surfaces surrounding the E appliance compared with the SL appliance (E >SL), differences were nearly significant ($P = 0.052$) for the maxillary arch but not for the mandibular arch ($P = 0.248$). The difference observed for the maxillary arch might be significant but does not meet the theoretical threshold of significant differences ($P < 0.050$). Also at T2, with intra-arch bracket pair comparisons, there appeared to be no statistically significant differences in the numbers of arches where plaque bacteria was higher on tooth surfaces surrounding the E appliance compared with the SL appliance. The higher *P* values might have been affected by the reduced statistical power because of fewer patients who completed the study and participated at T2 (n = 14) compared with more patients who began the study and came at T1 (n = 18).

In addition, when examining oral streptococci for the intra-arch comparisons, we found higher levels of streptococci in plaque surrounding the E bracket vs the SL bracket at both T1 ($P = 0.007$) and T2 ($P = 0.025$) (Fig 3). These results are consistent with the comparison of the means described in Table II. Thus, the use of E appliances promotes higher retention of plaque bacteria, including oral streptococci, at 1 week and potentially through 5 weeks after bonding.

Higher ATP-driven bioluminescence levels were observed in plaque surrounding E appliances. Figure 3 illustrates the intra-arch bracket pair comparisons divided on the basis of those with higher levels of ATP-driven bioluminescence from plaque on teeth bonded with an E vs an SL bracket. Histogram bar graphs are shown as numbers of arches where ATP-driven bioluminescence from plaque surrounding the E bracket was greater than corresponding RLU values from plaque surrounding the SL bracket (E >SL), as well as the reverse comparison (SL >E).

Like the data examining bacterial cell numbers, using intra-arch bracket pair comparisons, teeth bonded with E brackets compared with SL brackets generally had higher ATP-driven bioluminescence values from plaque. Specifically, more patients (and their arches) had higher ATP-driven bioluminescence values from plaque surrounding the E appliances compared with SL appliances (E >SL comparison) at T1 ($P = 0.028$, Fig 3). Thus, for bacterial cell numbers, higher ATP-driven bioluminescence values were found in plaque surrounding the E appliances compared with the SL appliances.

In most patients (or their arches), those who were placed in the E >SL category for total bacteria were also found in the E >SL category for streptococci and for ATP-driven bioluminescence. There were some exceptions, however, when subjects in the E >SL category for total bacteria were found in the SL >E category for either oral streptococci or ATP-driven bioluminescence.

When these exceptions were excluded from the data set, we still found statistically higher levels of bacterial load and ATP-driven bioluminescence in plaque surrounding the E appliances ($P < 0.05$), consistent with the conclusions when analyzing the complete data set.

There was high statistical correlation linking ATP-driven bioluminescence to total oral bacteria and total oral streptococci. By using plaque specimens from all patients, serial dilution plating of each specimen was conducted for quantification of total plaque bacteria with enriched medium (blood agar) and total streptococci with selective medium (mitis salivarius agar). When ATP-driven bioluminescence values were determined and compared with bacterial cell numbers, significant Pearson correlation coefficients of 0.800 and 0.689 were determined for total plaque bacteria and total plaque streptococci, respectively (1.0 = perfect correlation). When scatter plot analyses were conducted correlating total plaque bacteria with total plaque streptococci, increasing numbers of total plaque bacteria were found to track linearly with total plaque streptococci in a highly significant relationship ($r = 0.897$). When these ATP bioluminescence readings were analyzed by using the combined set of plaque and saliva specimens, highly significant correlation coefficients of 0.895 and 0.843 were found for total oral bacteria and total oral streptococci, respectively. When scatter plot analyses were conducted correlating total oral bacteria with total oral streptococci by using the combined plaque and saliva data set, increasing numbers of total oral bacteria were found to track almost linearly with total oral streptococci in a highly significant relationship ($r = 0.940$).

Thus, ATP-driven bioluminescence is highly predictive of total oral bacteria and, by statistical extension, also reflects the numbers of total oral streptococci in clinical specimens.

DISCUSSION

Iatrogenic decalcification of tooth enamel and the development of visible white spot lesions are undesirable and unfortunate consequences of fixed orthodontic therapy, potentially undermining the esthetic benefits often achieved through correction of the malocclusion. It is well documented that fixed appliances increase bacterial plaque accumulation and the risk for white spot lesions.[1-7,10,11,21] The development of the acid-etch bonding technique and the subsequent orthodontic application via the bonding of brackets in lieu of full banded appliances have not only facilitated the efficiency of orthodontic appliance construction, but also reduced the amount of tooth surface covered with appliances.[22] Nonetheless, bonded orthodontic brackets hinder access for good oral hygiene and create microbial shelters, resulting in the accumulation of plaque. The appliance architecture—specifically, the archwire ligation method—is an additional factor influencing bacterial colonization. Our results indicate that, in most patients, the teeth bonded with the SL appliance had fewer bacteria than those bonded with the E appliance.

Acid-producing bacteria colonize the tooth surface around orthodontic appliances, leading to enamel demineralization and often altering the appearance of the enamel surface.[1-7] Gorelick et al[1] and Mizrahi[5] found maxillary incisors and first molars to have the highest prevalence of white spot lesions. Gorelick et al[1] found that the maxillary lateral incisors had the highest incidence for white spot lesions, with the second most commonly affected teeth the maxillary central incisors. Interestingly, they found that length of treatment had little effect, with patients in treatment for 12 to 16 months experiencing the same incidence of white spot lesions as those with longer treatment schedules for up to 36 months.

Because of these potential side effects, several approaches have been recommended to help prevent the accumulation of plaque bacteria and subsequent enamel damage around fixed appliances. Fluoride-releasing compounds[23-26] and fluoridated elastomers[27,28] have been introduced, with questionable success for sustained enamel protection. Home-care regimens, such as daily sodium fluoride mouth rinses, have demonstrated significant measures of protection but suffer from potential noncompliance; this requires additional cooperation from patients for maximum effectiveness.[2,29,30]

In a scanning electron microscopic histologic study, Sukontapatipark et al[15] found the area arond the bracket base almost completely covered with a thick accumulation of bacteria in 1 week after placement of the appliances and attributed this to excess composite, with adjacent smooth areas exhibiting a less mature monolayer of bacteria. In similar studies, Glatz and Featherstone[31] and O' Reilly and Featherstone[2] demonstrated measurable histologic decalcification (up to 15% demineralization, to a depth of 50-75 μm) around orthodontic appliances after only a month of placement. Furthermore, O'Reilly and Featherstone[2] found that decalcification related to bonded orthodontic appliances occurred immediately around the appliance and not farther away along the buccal surface.

Based on observations that the maxillary lateral incisors have the highest prevalence of white spot lesions, ostensibly on the facial tooth surfaces at the immediate

periphery of the brackets, we used a circum-bracket plaque collection technique, a modification of the method of Forsberg et al.[10] We wanted to analyze the hygienic effects of the bracket ligation technique by studying and sampling plaque from tooth surfaces most affected and esthetically relevant, the anterior esthetic dentition.

The bonding of fixed orthodontic appliances hinders good oral hygiene and creates new shelters for microbial colonization. During treatment, there is demonstrated increased retention in the amounts of *Streptococcus mutans* and lactobacilli in saliva and dental plaque.[10,11] Several studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status, but few have evaluated the manner of ligation as an additional factor.[10,14,15] Two studies lacked randomization by using a split-mouth design[14,15] or lacked archwire engagement because only 1 tooth was bonded in each quadrant.[15] Randomization is important because hygiene studies have noted differences in brushing habits relative to handedness.[32,33] Right-handed people (and vice versa for left-handed people) tend to brush better or spend more time brushing their contralateral sides. Furthermore, no studies have compared differences in bacterial retention between traditional E vs SL methods.

Forsberg et al[10] studied the effect of microbial plaque retention around fixed appliances ligated with steel ligatures and elastomeric ties in 12 patients. Using circum-bracket sampling techniques, they found that the maxillary lateral incisors that were attached to archwires with elastomeric rings had more bacteria than incisors ligated with steel wires. They recommended avoiding the use of elastomeric ligatures in patients with poor oral hygiene because elastomeric ligation rings can significantly increase microbial accumulation on tooth surfaces adjacent to the brackets, leading to predisposition for dental caries and gingivitis. In contrast, Tukkahraman et al[14] found no significant differences in the numbers of microorganisms from teeth ligated using similar techniques, with either elastomeric rings or steel ligature wires. However, this study design was different from that used by Forsberg et al,[10] in that second premolars, not lateral incisors, were sampled, and the allocation of brackets was not randomized. Thus, Tukkahraman et al[14] used less commonly affected and less visible posterior teeth in their study, making sampling difficult because of the short clinical crowns and gingival proximity. Furthermore, the method of plaque collection was qualitative, not the quantitative, circum-bracket technique used by Forsberg et al[10] and in our study. These contrasting study designs, an the different statistical analyses, might account for the differences in conclusions in these studies.

Based on the results of Forsberg et al,[10] which indicated reduced bacterial retention around brackets ligated with steel ligatures as opposed to elastomeric ties, it was a logical hypothesis that the complete absence of a ligature—a self-ligating mechanism—would presumably be equally as hygienic, if not better than a stainless steel ligature. In our study, the most common method of archwire ligation, elastomeric ties, was chosen as the basis of comparison against the SL mechanism. The placement of steel ligatures on all brackets is time consuming and rarely done routinely in most orthodontic practices. Further studies should be done to compare the difference between steel ligatures and SL brackets.

The subjects we studied included some who maintained exquisite oral hygiene, with minimal plaque retained around the appliances, regardless of appliance type. Although this is desirable and encouraged for all our patients, it might not represent the oral hygiene of patients outside well-controlled clinical trials. Inclusion of patients with inadequate oral hygiene could diminish the true differences in plaque retention between the appliances. It would be interesting to perform a similar study including patients with inadequate oral hygiene, who are most affected by and at risk for decalcification. Additionally, the study was described to patients at the time of the informed consent, when they became aware of their inclusion in the study; thus, prior knowledge of their inclusion might have had a confounding behavioral effect on their level of oral hygiene.

The purposes of this longitudinal clinical study with a 4-quadrant, split-mouth design were to measure and compare the numbers of bacteria in plaque surrounding 2 distinct orthodontic brackets, SL vs E. Based on the observations that the maxillary lateral incisors have the highest prevalence of development of white spot lesions, ostensibly on the facial tooth surfaces at the immediate periphery of the brackets, we used a circum-bracket plaque collection technique, a modification of the method of Forsberg et al.[10] Although the results indicate fewer plaque bacteria surrounding the SL appliances, any mechanics with elastomeric chains or similar auxiliaries with SL appliances will presumably negate the beneficial effects of SL appliances, possibly also diminishing other purported benefits, such as reduced friction and lower force delivery. Thus, clinicians should consider this when placing elastomerics over the SL appliances to satisfy patient desires for colored bands or when elastomeric chains are to be in place for extended periods of time, as in space-closing mechanics. Moreover, although the results of this 5-week study reflect reduced plaque retention around the SL brackets, longer clinical trials should be conducted to

gain a better understanding of the clinical significance of different ligation methods.

This study has also provided validation that ATP-driven bioluminescence can be used as a potential quantitative biomarker of total plaque bacteria and streptococci that could be rapidly and reliably measured at the chair-side. This study has broad implications in dentistry and can be used translationally in the clinic to monitor the effectiveness of oral hygiene during orthodontic treatment and to potentially determine the efficacy of interventional therapies for dental caries, enamel decalcification, and white spot lesions.

CONCLUSIONS

1. The results of this study indicate that SL appliances promote less retention of oral bacteria, including streptococci, compared with E appliances. In most patients, the teeth bonded with an SL attachment had fewer bacteria in plaque and lower levels of ATP bioluminescence than did the teeth bonded with an E bracket.
2. ATP-driven bioluminescence values correlated significantly to numbers of oral bacteria and oral streptococci ($r = 0.895$ and 0.843, respectively), indicating that ATP-driven bioluminescence might serve as a useful tool in the rapid, chair-side quantification of bacterial load and in the assessment of oral hygiene during orthodontic treatment.

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