Self-ligation vs. elastomers: One-year follow-up study examining bracket archwire ligation technique on microbial colonization and white spot lesion formation

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ABSTRACT

Introduction: Formation of white spot lesions (WSL) is a common risk associated with fixed orthodontic appliances. The maintenance of good oral hygiene is made difficult by orthodontic brackets which results in bacterial plaque accumulation, increasing the risk of WSL. There are several studies which have investigated the effects of fixed orthodontic appliances on the microbial flora profile; however, few studies have compared differences between conventional and self-ligating brackets as well as obtain a quantitative estimate of the plaque. Several studies have evaluated enamel demineralization photographically, but none have compared differences between white spot lesions associated with conventional versus self-ligation appliances. Several studies have also used laser fluorescent detection of white spot lesions *in vitro* but, few clinical studies have been done with the DIAGNOdent evaluating WSL surrounding brackets. Objectives of this prospective longitudinal study, conducted as a 1-year follow-up study, were to (1) compare plaque bacteria amounts at the bracket-tooth interface with use of elastomeric-ligating (EL) vs. self-ligating (SL) brackets, (2) measure bacteria after initiation of elastomeric chain use, (3) evaluate use of ATP-driven bioluminescence for quantification of total oral bacteria, and (4) analyze formation of WSL by photographic evaluation and laser light fluorescence (DIAGNOdent).

Methods: Thirteen subjects had full fixed appliance orthodontic treatment where upper and lower lateral incisors were bonded with either an EL or SL brackets. A total of 50 teeth were included in the study (1 subject with only upper laterals included). All other teeth received self-ligating brackets. Plaque samples were collected from facial surfaces of the incisors at 1 year and then 5 weeks later after use of elastomeric chains. Total plaque bacteria were quantified using standard plating methods and by direct measurement of ATP-driven bioluminescence. To

evaluate WSL, standardized photographs and DIAGNOdent measurements were analyzed. A 2 X 2 X 2 X 2 mixed-design full factorial ANOVA was conducted to determine significant patterns of differences between EL and SL brackets.

Results: There were no statistical differences in bacterial numbers surrounding the EL vs. SL brackets. ATP-driven bioluminescence values for teeth with EL (GM= 8.192E+07) and SL (GM= 6.950E+07) brackets were similar at 1 year. After five further weeks of elastomeric chain, ATP-driven bioluminescence values increased for teeth with SL brackets, occurring in the maxillary arch only (GM=1.298E+08), with no change observed for teeth fitted with EL brackets (GM= 6.726E+07). ATP-driven bioluminescence values correlated to the numbers of total oral bacteria ($r=0.85$; $p<0.05$). DIAGNOdent measurements were found to have low sensitivity (0.45) and good specificity (0.82) when compared to WSL determined using photographic evaluation.

Conclusions: After one year of treatment there were no differences in retention of plaque bacteria or WSL comparing the two bracket types. When elastomeric chains were introduced, in the upper arch only, SL brackets were associated with the retention of higher amounts of plaque bacteria than were EL brackets. ATP-driven bioluminescence values were significantly correlated to the numbers of total oral bacteria. The use of the DIAGNOdent, compared to visual-photographic evaluation, may be of limited utility when evaluating WSL surrounding brackets.

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INTRODUCTION

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 (WSL) is a common undesirable side effect. In the clinical setting, trying to prevent the formation of WSL in patients being treated with orthodontic appliances can be a daunting task. Numerous studies have shown that there is an increase in caries-causing bacteria when fixed orthodontic appliances are placed (Ahn et al. 2005, Anhoury et al. 2002, Bollen et al. 2008, Forsberg et al. 1991, Naramjo et al. 2005, Ristic et al. 2007, Sudjalim et al. 2006, Turkkahraman et al. 2005). Due to increased difficulty in cleaning around orthodontic appliances, there is an increase in plaque accumulation and in the development of WSL (Ahn et al. 2005, Aljehani et al.2004, Forsberg et al 1991, Gorelick et al. 1982, Staudt et al. 2004, Sudjalim et al. 2006, Sukontapatipark et al 2001, Turkkahraman et al. 2005). For example, Gorelick and colleagues (1982) found an increase in the prevalence of at least one WSL after orthodontic bracket removal in 50% of treated patients as compared to 24% of untreated subjects.

The oral cavity is home to over 800 different species of bacteria. The mouth contains many niches which allows for such a diversity of inhabitants. Bacteria considered most significantly involved with carious lesions include *Streptococcus mutans*, *Actinomyces* and *Lactobacillus* species. These cariogenic bacteria can cause enamel demineralization (WSL and subsequent cavitation) via the accumulation of acid bi-products, most notably lactic acid, as a result of the metabolism of simple carbohydrates (Ahn et al. 2005, Anhoury et al. 2002, Barberia et al. 2008, Sudjalim et al. 2006, Turkkahraman et al. 2005). Metal brackets have been found to enhance the presence of *S. mutans*, which are considered among the first to colonize brackets

(Ahn et al. 2005, Anhoury et al. 2002). Thus, with increased accumulation of plaque due to orthodontic appliances, there is an increase in both total as well as cariogenic bacteria.

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 built into the bracket). Studies have evaluated the microbial colonization of conventional brackets associated with ligation wires versus elastomeric modules and while some found no significant difference in plaque accumulation (Sukontapatipark et al. 2001, Turkkahraman et al. 2005), others found increased plaque accumulation with the use of elastomeric-ligation (Forsberg et al. 1991, Pellegrini et al. 2009). To date little has been published evaluating the quantity of plaque bacteria around self-ligating versus conventional brackets. Recently, Pellegrini and collaborators (2009) quantified plaque bacteria around self-ligating versus conventional elastic-ligating brackets and found that at five weeks post-bonding there was significantly lower plaque accumulation around self-ligating brackets. The vendors for self-ligating brackets and many orthodontists who have switched from conventional to self-ligating brackets claim reductions in the amount of plaque accumulation, but further studies are required to substantiate such claims ((Harridine 2003). With the putative reduction in accumulation of plaque, suggestions have been made that self-ligating brackets will also promote reductions in the development of WSL. Findings by Pellegrini et al. (2009) provide suggestive evidence that reduction in WSL may indeed be a possible benefit of self-ligating brackets, however, they concluded that further studies are necessary, including longer periods of evaluation and larger sample sizes.

Methods for documenting demineralization and WSL formation include digital photography and laser-light fluorescence. Digital photographs are an inexpensive and

convenient method of evaluating the changes in the appearance of tooth surface enamel over time. Photographs made from multiple angles can be used to evaluate the facial surfaces. In addition, images can be archived and subsequently evaluated in random sequence, allowing for assessor blinding and an unbiased detection of developing WSL (Benson et al.2003, 2004). Although useful for evaluating advanced demineralization, significant mineral loss in enamel is required before WSL are readily visible to the eye (Staudt et al. 2004). Early mineral loss can be detected by use of laser-light fluorescence such as the DIAGNOdent (Kaltenbach & Voigt Gmbh & Co., Biberach, Germany, Distributed by KaVo America Corp., Lake Zurich, IL), a portable red laser-light fluorescent detector. Several studies have used the DIAGNOdent to perform *in vitro* studies of WSL with brackets, but few have used this detection method in subjects undergoing fixed orthodontic treatment (Aljehani et al. 2004, Barberia et al. 2008, Pinelli et al. 2002, Staudt et al. 2004).

LITERATURE REVIEW

Plaque/ Microbes

The occurrence of white spot lesions (enamel demineralization) around orthodontic fixed appliances is common during orthodontic therapy (Ahn et al. 2005, Anhoury et al. 2002). The placement of bonded brackets creates a favorable environment for accumulation of microorganisms that cause demineralization. Metal brackets produce a change in the oral environment, increasing plaque accumulation and decreasing the pH. Ahn et al. found that cariogenic streptococci strains have a characteristic adhesion pattern to metal brackets; *S. mutans* OMZ65 was found to adhere to the bracket surfaces more significantly than other strains (*S. mutans* LM7, *S. sobrinus* 6715, *S. sobrinus* B13) in the study, regardless of the incubation time.

Ahn et al. also found that increased incubation times caused an increase in the level of bacterial adhesion; however, salivary coating of the metal brackets caused a significantly smaller increase in the level of bacterial adhesion compared to non-salivary coated brackets. Ahn et al. results show that adhesion amounts of cariogenic streptococci to metal brackets may be dependent on strains present in the oral cavity and the quality/quantity of saliva coating. A study by Anhoury et al. found an increase in plaque and levels of *S. mutans* and *Lacobacillus* species to be similar on both metal and ceramic brackets.

The placement of fixed appliances causes an inflammatory reaction of gingival tissues and can cause a significant change in the bacterial composition of subgingival plaque. The prospective study by Ristic et al. included 32 patients in which the health status of periodontal tissues was determined using periodontal indices (Plaque index system, gingival index system, and gingival bleeding index system) along with assessment of probing pocket depths and micorbiological examination of subgingival bacterial plaque. Periodontal measurements were taken initially, three weeks prior to appliance placement (T0) and at one (T1), three (T3), and six (T6) months post-orthodontic appliance placement. The subgingival plaque samples were collected using two sterile paper points inserted into the deepest part of the sulcus for sixty seconds at T0, T1, T3, and T4. Microbial analysis was performed using culture techniques to confirm the presence or absence of periodontopathic anaerobes (*P. intermedia, A. actinomycetemcomitans, P. gingivalis, and F. nucleatum*).

The periodontal indices performed showed minimum values at T0 with maximum values at T3 followed by a slight decrease at T6. Thus, there was an increase in gingival inflammation following bracket placement up to three months with a subsequent decrease by six months into treatment (Ristic et al. 2007). The periodontal index findings were found to be associated with

the microbial assessment findings; the occurrence periodontopathic anaerobes was lowest at three weeks prior to appliance placement (T0) and highest at three months post-appliance placement (T3), with a subsequent decrease by six months post-appliance placement (T6). Ristic et al. suggest that the decrease in periodontal index and microbial findings between T3 and T6 could be explained by reestablishment of host-microrganisms balance. The results show an increase in gingival inflammation and infection of periodontopathic anaerobes following placement of orthodontic fixed appliances. In a similar study by Naranjo et al., authors found an increase in gingival inflammation and periodontal pathogens as well as superinfecting microorganisms such as *Enterobacter cloacae, Klebsiella oxytoca, K. pneumonia,* and *Serratia marcescens*. It is thus important to observe patients regularly and provide continuous good oral hygiene instruction with constant reinforcement during treatment utilizing fixed orthodontic appliances.

The colonization of bacteria associated with fixed orthodontic appliances is influenced by "design and surface characteristics of both orthodontic attachments and composite" (Sukontapatipark et al. 2001). With the use of scanning electron microscopy (SEM), Sukontapatipark et al. evaluated the accumulation of bacterial plaque at the tooth-bracket junction. Sukontapatipark et al. also compared differences in accumulation between brackets ligated with steel wires and elastomeric rings. Nineteen subjects requiring orthodontic treatment with extraction of two or four premolars participated in the study. A preliminary study using a total of 32 extracted teeth was performed and consisted of two groups: (1) teeth with naturally grown plaque without bonded brackets were assessed and (2) teeth were cleaned after extraction, then etched and brackets were bonded. The experimental study consisted of three groups according to the time between bonding and extraction: 1-, 2-, 3-week groups. Extractions were

carefully performed so as not to disrupt plaque on the labial surfaces of the teeth or cause bracket debonding. To remove blood and debris, teeth were rinsed immediately after extraction. The buccal surfaces of the teeth were disclosed for plaque attachment and photographed for documentation. Teeth were prepared for SEM examination. SEM was used to assess plaque composition based on morphological characteristics.

In Sukontapatipark et al.'s preliminary study, the non-bonded extracted premolars showed a fine layer of plaque corresponding to the stained enamel surface. With higher magnification of the surface, clumps of cocci and rods aggregated in areas with surface irregularites and an extracellular amorphous matrix. The cleaned and bonded extracted premolars showed excess bonding composite around the bracket base. With higher magnification, the bonding adhesive was found to be rough and "a gap at the composite-enamel junction was present along the edge" (Sukontapatipark et al. 2001). In the one week specimens, a discrete layer of cocci and a few short rods were seen in the cervical region and lateral to the brackets on the enamel surfaces. Large bacterial deposits were found underneath bracket wings, ligatures, and on composite surfaces. The composite-enamel narrow gap was harboring numerous bacteria in all specimens. In the two week specimens, most "presented features of young plaque consisting of cocci and rods embedded in and inter-microbial matrix" (Sukontapatipark et al. 2001). There was an increased presence of organisms that are characteristic of mature plaque. Again, "a gap with lodged bacteria along the periphery of the composite" was found similar to week one specimens (Sukontapatipark et al. 2001). In the three week specimens, color photographs showed abundant plaque on the appliances, excess composite, and gingival and lateral to the brackets. SEM revealed a fine layer of plaque on the enamel and a thick layer of plaque on the excess composite, brackets, and ligatures.

In Sukontapatipark et al.'s study, the brackets ligated with stainless steel wires appeared to retain less plaque than those ligated with elastomeric rings. At higher magnification, cervical plaque consisted of "microcolonies of cocci and short rods embedded in a prominent inter-microbial matrix in both groups of ligation" (Sukontapatipark et al. 2001). Composite surface plaque had a more mature composition with predominant filaments together with some rods and cocci. Bracket and ligature surfaces also had bacterial colonization. The gaps at the composite-enamel junction were conspicuous due to the abundance of bacteria present.

Within one week of bracket placement, the number of bacteria present increases significantly with a monolayer forming on the enamel gingival to the composite; therefore,"...Excess composite is an important predisposing factor for plaque accumulation" (Sukontapatipark et al. 2001). The findings show that plaque accumulation occurs primarily lateral and gingival to the bracket base corresponding to the areas in which the majority of white spot lesions occur. Sukontapatipark et al. showed that neither ligation method seemed to "affect the distribution of bacterial morphotypes on both composite and enamel surfaces".

Development of dental plaque is multifactorial, depending on the individual's diet, oral hygiene, quality of saliva, and oral bacterial composition (Forsberg et al. 1991). Along with bracket design, orthodontic attachments, and the composite used, Forsberg et al. suggest that the method of ligation use to secure the archwire is another important factor that influences plaque retention during orthodontic treatment. In the study by Forsberg et al., microbial colonization of *S. mutans* and *Lactobacilli* was assessed in association with two ligation methods: steel ligature wires or elastomeric rings. Twelve subjects requiring orthodontic therapy were enrolled in the study. Following bracket bonding, one side of the midline was randomly selected to have elastomeric rings used and the other side steel ligatures. Plaque samples were collected at 4, 10,

19, 34, and 61 weeks using charcoaled points on the labial surface of upper lateral incisors around the circumference of the brackets. Saliva samples were collected prior to bonding, at each plaque collection time point, and 6 weeks post-orthodontic therapy. The method of ligation was switched at each collection time with the opposite side of the midline: the side that was previously tied with elastomeric rings was changed to the side tied with steel ligatures and vice versa. Microbiological studies were performed with the plaque and saliva samples using cultivation analysis for total bacteria and *S. mutans/Lactobacilli*. From the plaque samples, patients were assigned to one of two groups: those who exhibited the highest number of bacteria on the lateral incisor tied with an elastomeric ring and those who exhibited the highest number of bacteria on the lateral incisor tied with a steel ligature. Forsberg et al. found that the majority of the subjects exhibited a greater number of bacteria in plaque on the teeth tied with an elastomeric ring. A significant difference was found showing that ligation with elastomeric rings was associated with higher mean numbers of bacteria at every collection time point than ligation with steel wires. The salivary samples show an increase in both *S. mutans* and *Lactobacilli* from prior to treatment when compared to those found during treatment. The salivary samples also showed that the numbers of these bacteria return to pretreatment levels once the appliances are removed.

Turkkahraman et al. (2005) performed a study that evaluated the effect of microbial colonization on ligation method of orthodontic fixed appliances. The study included twenty one subjects requiring orthodontic therapy between the ages of 11 and 25 with full dentitions. The study design included a split-mouth design in which the brackets in the upper arch on the right side were ligated with elastomeric rings and the left side were ligated with stainless steel ligature wires. Periodontal measurements and microbial samples were taken prior to bonding (T0), one week after bonding (T1), and at five weeks after bonding (T2). Once ligatures and wires were

carefully removed, plaque samples were taken from upper second premolars around the circumference of the brackets. Samples were cultivated and analyzed by bacterial plating analysis for total bacteria, *S. mutans*, and *Lactobacilli* numbers. Periodontal evaluations included "the gingival index (GI), bonded bracket plaque index (BBPI), bleeding on probing (BOP) values, and pocket depth (PD)". Turkkahramn et al. (2005) found that, though microbial counts were slightly higher in the elastomeric group, there was no significant difference in the bacterial counts. There was a significant increase in total bacteria, *S. mutans*, and *Lactobacilli* counts between all time points. Periodontal findings were insignificant except that at T2 there were significantly more teeth with BOP in the elastomeric group. Thus, contrary to Forsberg et al. findings, Turkkahraman et al. (2005) found no significant difference in bacterial numbers between the two archwire ligation techniques: elastomeric rings vs. ligature wires.

White Spot Lesions

Imbalance between demineralization and remineralization may result in loss of tooth mineral content leading to white spot lesions and cavitation (Barberia et al. 2008). Demineralization of enamel occurs prior to the appearance of white spot lesions. Methods of early detection have been developed and include photographic analysis, digital imaging fiber-optic transillumination (DIFOTI), electronic caries monitor, quantitative laser/light-induced fluorescence (QLF), and red laser light induced fluorescence (DIAGNOdent) (Aljehani et al. 2004, Staudt et al. 2004). Red laser-light induced fluorescence may be easier to use than other methods and measurements are given immediately upon use (Staudt et al. 2004).

Photographic WSL Assessment

Photographic assessment of white spot lesions with the aid of computer software analysis has been shown to be reproducibly quantifiable (Benson et al. 1998, Benson et al. 2000, Benson

et al. 2003, Livas et al. 2008). In a study by Benson et al. (2003), standardized photographs were taken with the use of a positioning jig. The jig consisted of a full size rectangular wire bent perpendicular to the bracket slot and held in the bracket with an elastomeric tie. The jig allowed for horizontal orientation and standardized distance while photographs were taken of the study teeth. Computer software analysis was found to be reliable with perpendicular photographs and photos up to 20° above or below perpendicular (Benson et al. 2003, Livas et al 2008.). Benson et al. (2004) also evaluated disclosed plaque around brackets in a clinical study using photographic assessment. Again, a photographic jig was used as previously described. Benson et al. (2004) aimed to evaluate the effect of fluoridated elastomers on plaque around orthodontic brackets *in vivo*. In this study, a fluoridated elastomer was randomly placed around brackets on either the upper right incisors and lower left canine, or the upper left incisors and lower right canine. Evaluators were blinded to prevent bias and brackets were blocked out of the digital photos prior to evaluation to exclude plaque located on the bracket, rather that the surface of the tooth (Benson et al. 2004).

DIAGNOdent WSL Assessment

An *in vivo* study of red laser-light fluorescence as a diagnostic method was evaluated on smooth surfaces of teeth with the use of the DIAGNOdent by Pinelli et al. Fifty subjects were enrolled in the study with a total of 220 lesions evaluated. Lesions were clinically evaluated by visual inspection with mouth mirrors and reflector light and categorized as either active or inactive. The lesions were evaluated independently with the DIAGNOdent recording the highest numbers ranging from 0 to 99. Cut off points were established as values 0 to 4 for arrested lesions and 5 to 99 for active lesions. Teeth were cleaned thoroughly, dried for 10 seconds, and then the laser device was applied to the surface. One week later the same measurements were

repeated under identical conditions to evaluate possible measurement errors. Inter- and intra-examiner reproducibility were in agreement according to clinical and laser examinations. The validity of the laser examination according to clinical evaluation of active and inactive lesions was determined with a sensitivity of 0.72 and specificity of 0.73 (Pinelli et al. 2002). A study evaluating occlusal surfaces with the DIAGNOdent found a sensitivity of 0.79 and specificity of 0.87 (Barberia et al. 2008). This study however, included primary molars which are considered to have thinner layers of enamel. Different cut-offs were also used for occlusal measurements with the DIAGNOdent which may explain differences in sensitivity and specificity. Though 28% of lesions requiring treatment were not diagnosed properly and 27% of lesions were false positives, these findings show relatively good sensitivity and specificity can be used in addition to other clinical diagnostic methods (Pinelli et al. 2002).

Staudt et al. performed an *in vitro* study evaluating the use of the DIAGNOdent around brackets in an attempt to determine whether brackets or the bonding process interfere with readings. Thirty extracted human molars were selected each with at least one smooth surface decalicification present. The red laser-light fluorescent measurements ranging from 0 to 99 were taken with the DIAGNOdent using its tapered tip (tip A). The bracket and composite to be used in bonding were measured with the DIAGNOdent to determine fluorescence of these materials. Four separate measurements were recorded on each tooth: prebonded enamel, etched enamel, bonded enamel (brackets bonded), and debonded enamel (after bracket and composite removal). The measurement sights were standardized on each tooth to ensure measurements from the same locations. Staudt et al. found that the measurements for prebonding and after etching showed no statistically significant differences. However, after bracket bonding the fluorescence showed statistically significant decrease by a median value of 0.5 units compared to prebonding. After

debonding, fluorescence was found to increase by a median value of 0.5 units and 1.0 units compared to prebonded conditions and bonded conditions respectively. Though, Staudt et al. found some statistical significance between after bonding and debonding compared to prebonding measurements, the values were rather small and are not considered clinically significant. This is due to a large range of values (0 to 99) with cut –off values assigned for specific stages of demineralization. Thus, with clinical use of the DIAGNOdent, measurements may be taken around bonded brackets.

In a study by Gorelick et al., researchers evaluated the frequency of the occurrence of white spot lesions and the susceptibility of different teeth within the oral cavity. In a control group of fifty children, they found a 24 percent prevalence of at least one tooth with a white spot lesion. However, out of all the teeth examined (1,006), only 3.6 percent had white spot lesions (Gorelick et al. 1982). Out of one hundred twenty one debonded orthodontic patients, researchers found a 49.6 percent prevalence of at least one tooth with a white spot lesion (Gorelick et al. 1982). Interestingly, Gorelick et al. found the incidence of white spot lesions for boys was 44 percent and 54 percent for girls. Though the prevalence of orthodontic patients with at least one white spot lesion was high, only 10.8 percent of the 2,211 teeth examined had a white spot lesion (Gorelick et al. 1982). The frequency of white spot lesions among individual teeth showed significant differences. Gorelick et al. found the most affected tooth was the maxillary lateral incisor (23 percent), and then the mandibular canine (18 percent), the least affected tooth was the maxillary first molar (only 1 percent). The segments most affected were the mandibular posterior (15.3 percent) and maxillary anteriors (14.1 percent) (Gorelick et al. 1982). The findings by Gorelick et al. show that white spot lesions are indeed an orthodontic problem and certain teeth are at higher risk for white spot lesion formation. Gorelick et al.

suggests that, due to their findings, the predisposition to the formation of white spot lesions may be due to the access of salivary flow and the inter-gingival-bracket distance. More research is obviously needed to determine salivary flow's relationship to white spot lesion formation.

The purposes of this prospective longitudinal study were: 1) To measure and compare the levels of total plaque bacteria on the tooth surface at the periphery of the bracket-tooth interface of EL vs. SL orthodontic brackets after one year of treatment, 2) to compare levels of plaque bacteria between the two bracket types after five additional weeks post-initiation of elastomeric chain use, 3) to evaluate the use of ATP-driven bioluminescence to rapidly quantify total oral bacteria, and 4) to analyze white spot lesion formation on surfaces surrounding both EL vs. SL brackets as determined by visual inspection and laser light fluorescence (DIAGNOdent).

HYPOTHESIS

Hypothesis 1: The total bacterial plaque collected from teeth bonded with SL orthodontic brackets will be less than the total bacterial plaque collected from teeth bonded with EL brackets.

Null hypothesis 1: There will be no difference in the total bacterial plaque collected from teeth bonded with either SL or EL brackets.

Hypothesis 2: After 5 further weeks of elastomeric chain use, there will be no significant difference in total bacterial plaque collected from teeth bonded with SL vs. EL brackets.

Hypothesis 3: ATP-driven bioluminescence values will correlate to with total bacterial plaque collected from the facial surface of teeth bonded with both bracket types.

Null hypothesis3: No correlation will be found between ATP-driven bioluminescence values and total bacterial plaque.

Hypothesis 4: WSL DIAGNOdent readings will correlate well with visual-photographic WSL.

Null hypothesis 3: There will be no correlation between DIAGNOdent WSL measurements and visual-photographic WSL.

MATERIAL AND METHODS

Patient Demographics and Appliance Placement: Thirteen out of fourteen subjects who finished the study by Pellegrini et al. (2009) were enrolled in this follow-up study (Table I; p. 44). The original criteria for selection in the Pellegrini et al. (2009) study were 12 years of age or older at the start of treatment (mean=13.9; range 12.1 to 17.2) and demonstration of good oral health. All patients selected were diagnosed as requiring fixed appliance orthodontic treatment and were not missing any lateral incisors. Patients who were pregnant, diabetic, using mouth rinses or interacting medications, including antibiotic therapy within three months prior to the study, were excluded. One subject (patient #13) who finished the Pellegrini et al. (2009) study dropped out of the continuation 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 lefthandness (1 of only 3 left-handed individuals in the study) and received only maxillary appliances (1 of only 2 individuals in the study). In addition, patient #8 did not complete the five further weeks of elastomeric chain use. All patients were treated at the Oregon Health & Science University (OHSU) Department of Orthodontics, and the OHSU Institutional Review Board (IRB) approved the human subjects protocol prior to the initiation of the study. Participants and their parent/guardian were assigned study identifier numbers that were accessible only to the investigators in the Departments of Orthodontics and Integrative Biosciences (David Covell, Jr. Ph. D., DDS, Peter Pellegrini, DDS, Tyson Buck, DMD, Curtis A. Machida, Ph. D. or Tom Maier, Ph. D.).

A consent form for routine orthodontic care, currently in use in the OHSU Orthodontic clinic, was obtained, and subjects selected for this study read and signed a second consent form specifically for the research study that was presented to the participant and parent/guardian. The participant's oral health was initially assessed and reviewed by an orthodontic resident, under the

supervision of an attending faculty member. We requested that the participants refrain from the use of any additional oral hygiene aids, such a fluoridated or medicated mouth rinses, and also refrain from eating or drinking 1 hour prior to the sampling appointments. We used the OHSU Short Form for non-English speakers and obtain interpreters, if necessary, through the University or the School of Dentistry. A study investigator, interpreter, participant, and the participant's parent/guardian (if the participant is not his or her own legal guardian) were all present at the time of the participant's enrollment in the study. The interpreter translated and orally stated the OHSU Short Form and Written Summary of the Study to the participant and the participant's parent/guardian. All required signatures have been previously obtained. The study calls for no additional treatment or procedures not normally performed in the routine oral hygiene provided during initial bonding or orthodontic adjustment visits. This study proposes to use plaque which is normally discarded during the initial and any subsequent treatment visits.

The following information was taken from the participant's chart and determined from examination during the study visits: 1) Participant name, including age and gender, 2) Chart Number, 3) Sample Number, including collection date and time, 4) Present Medications, including fluoride tabs, oral rinses, and antibiotic use within the last 30 days, (this is done to ensure that participants initially included in the study that subsequently take an interacting medication were flagged and excluded from the study) 5) Last tooth brushing, 6) Hygiene/Tissue condition, including presence of gingivitis and periodontal disease, 7) Time since last meal/snack 8) Collection site comments and general comments. This information was collected on the Data Collection Sheet at one year and again at five further weeks after elastomeric chain wear (Figure 1; p. 50).

Tyson Buck, DMD completed a Data Collection Sheet for each participant. The doctor assigned for the plaque collection (Tyson Buck, DMD) utilized a standardized collection technique (Pellegrini et al. 2009), and was the sole person responsible for the sample collection. At the initial bracket bonding appointment, all teeth were polished with a coarse grade prophylaxis paste using a rubber cup and slow speed handpiece. The two different brackets were bonded to lateral incisors by allocation using a split-mouth design (Figure 2A; p. 50). Initially in each arch one lateral incisor received either an "experimental bracket" (self-ligating, 0.022" In-Ovation-R®, GAC International, Bohemia, NY [Figure 2B; p. 50]) or a "control bracket" (standard, elastomeric-ligating, 0.022" Mini-Ovation®, GAC [Figure 2C; p. 50]), and the contralateral incisor received the other type of bracket. The right-left distribution of EL vs. SL brackets was evenly distributed among patients. Lesaffree et al. (2008) discusses the methodological 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-handed and left-handed people tend to spend more time brushing on the contralateral side (Thienpont et al. 2001, Rugg-Gunn 1978). There were four males and nine females, and 10 subjects were right handed, two were left handed, and one was ambidextrous. The patient population as described above, including information concerning bracket-tooth assignments, can be found within Table 1 of the Pellegrini et al. (2009) study (see Table 1, page 44; modified from Pellegrini et al. 2009). The appliances were bonded using composite resin (Neo-bond®, GAC), with all but the assigned lateral incisors bonded with SL 0.022" brackets (In-Ovation-R®, GAC International, Bohemia, NY).

The amount of time between brushing and sample acquisitions at adjustment appointments ranged from 1-5 hour(s). Subjects were requested to refrain from eating or

drinking one hour prior to the sampling appointments and plaque was collected from all four of the lateral incisors with the exception of one patient (#5), where only the upper lateral incisors were included (had upper appliances only). At the sampling visits, the elastomeric modules were removed or the SL mechanism disengaged and the archwires removed. Plaque specimens were obtained from around the bracket base of each lateral incisor utilizing a sterilized dental scaler (# 8/9 Orban DE hoe scaler; Hu-Friedy, Chicago, Illinois [Figure 3A; p. 51]). A four-pass technique (Pellegrini et al. 2009) was employed where the investigator moved the instrument tip around the circumference of the bracket (Figures 3B and 3C; p. 51). To avoid overloading the instrument tip, separate passes were made along the enamel surface at the gingival, mesial, distal, and incisal of the bracket-tooth interface. For each tooth the four samples were placed into one anonymously coded tube and transported to the laboratory for analysis. The anonymous code identified both the tooth and the participant, assuring blinding of laboratory personnel. After plaque sampling, subjects were given a paraffin wax tablet that they were instructed to chew for 1-5 minutes to obtain a minimum of 5 ml of saliva, collected in a sterile, calibrated container.

Plaque and saliva samples were collected at an orthodontic adjustment appointment corresponding to 1-year of treatment. At this appointment after the archwires were ligated in the SL brackets, elastomeric chains were placed from first molar to first molar in both arches (except in the lower arch for the subject with only upper appliances). The patients were recalled five weeks later and plaque samples were again collected.

Microbiological Analysis of Samples (Figure 4; p. 51): Five samples (plaque samples from 4 teeth and a stimulated saliva sample) were collected per subject at each sampling appointment. Each plaque sample was diluted in 1 ml of 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 then underwent 10-fold serial dilutions in PBS and the dilutions were plated on enriched blood agar (PML Microbiologicals, Wilsonville, OR) to determine total bacterial numbers.

ATP-Driven Bioluminescence of Samples: ATP levels contained in bacteria from plaque specimens were determined with the use of the BacTiter Glo Microbial Cell Viability Assay kit (Promega, Madison, WI; product number G8231). ATP-driven bioluminescence, using the luciferin substrate and luciferase enzyme to generate light (Ronner et al. 1999) (Figure 5A; p. 52), was measured using Veritas Microplate luminometer (Turner Biosystems; San Diego, CA [Figure 5B; p. 52]). Relative light units (RLUs) were calibrated using a standard curve of ATP (pM concentrations or greater; powdered chemical obtained from Sigma Chemical, St. Louis, MO) and correlated against optical density (OD) (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 at the 1-year appointment following plaque collection, the lateral incisors were thoroughly cleaned and briefly air-dried. Photographic images were made using a standardized technique where a jig (Figure 6A; p. 53) was used to establish a 15 cm distance from the buccal surface of a tooth to the camera lens (Benson et al. 2003). Three images of the lateral incisors were made, one perpendicular to the buccal surface and two at approximately 20° above and 20° below perpendicular to the buccal surface as assessed by eye (Benson et al. 1998, 2000, 2004, Livas et al. 2008). The three photographic images were coded and archived for WSL evaluation. Evaluation for the presence of WSL was conducted and scored using a modified Gorelick index (Gorelick et al. 1982) using 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) (Figure 6B; p. 53). The photographs (e.g.: Figure 6C and D; p. 53) were used to correlate visual WSL formation to laser light fluorescence readings.

For laser light fluorescence (DIAGNOdent, KaVo America Corp., Lake Zurich, IL [Figure 7; p. 53]) readings, a technique similar to that of Staudt and associates (2004) was used. The sample teeth were isolated with cotton rolls, air-dried for a few seconds, and 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° to the tooth surface to allow measurement of the enamel closest to the bracket (Staudt et al. 2004). The largest unit (ranging 0-99) was recorded for each of the four sides. According to the manufacturer's recommendations, readings from 0-4 correspond to no lesion, 5-10 correspond to slight lesion, and 10-99 correspond to severe lesion. For evaluation of method error, a second set of readings were made on four subjects selected at random. DIAGNOdent method error was calculated using the Dalberg Formula:

$$s=\sqrt{\sum d^2/2n},$$

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, and distribution characteristics were calculated. The distributions for total bacterial numbers and ATP-driven bioluminescence determinations (in RLUs) were severely positively skewed. A natural logarithmic transformation of these variables was applied, which effectively normalized 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 their influence on the analysis. All statistical analyses were performed on the transformed variables, and presentation of means and standard deviations or standard errors will be on the back-transformed variables to bring values back to the original metric. Note that the back-transformation of log transformed variables provides geometric means and not arithmetic means.

We performed two 2 X 2 X 2 X 2 mixed-design full factorial ANOVAs to determine whether there were any significant patterns of differences in total bacteria and ATP-driven bioluminescence values between EL and SL brackets. The split-mouth design can be considered an adaptation of the split plot design that has its roots in agricultural research (Lesaffre et al. 2007). This design calls for a within-subjects analysis (repeated measures of teeth and teeth within persons are correlated), which allows for the removal of subject effects from the error term used in ANOVA, and thus increases the power of the analysis. The pattern of bracket assignment was the only between-subjects factor with two levels (EL/SL/EL/SL vs. SL/EL/SL/EL, with the order corresponding to the following teeth: maxillary right lateral incisor, maxillary left lateral incisor, mandibular left lateral incisor, mandibular right lateral incisor). Note that the between-subjects factor in this analysis is analogous to a sequence effect that is evaluated in a pure crossover design (Lesaffre et al. 2007). The remainder of the independent variables were within-subjects variables with two levels each: time (52 weeks vs 57 weeks which includes five additional weeks of elastomeric chain use), bracket assignment (EL vs SL), and arch (maxillary vs mandibular). Any significant interactions were plotted to facilitate interpretation. Significant interactions were not followed up with post hoc simple main effect tests given the high number of multiple comparisons that would need to be conducted and the relatively small size of the sample.

To explore the correspondence of DIAGNOdent WSL measurements with photographic-visual WSL indices, we calculated the sensitivity (number of correct DIAGNOdent positive WSL identifications/[number of correct DIAGNOdent positive WSL identifications + number of incorrect DIAGNOdent negative WSL identifications]) and specificity (number of correct DIAGNOdent negative WSL identifications/[number of correct DIAGNOdent negative WSL identifications + number of incorrect DIAGNOdent positive WSL identifications]) of DIAGNOdent measurements.

RESULTS

Findings of Plaque Bacterial Load: The raw mean bacterial numbers for total bacteria contained in plaque surrounding the EL and SL brackets are shown in Table II (p. 44). The results from the full factorial ANOVA are presented in Table III (p. 45). There were no significant differences in the numbers of total plaque bacteria surrounding the two different bracket types at either one year of treatment or after five subsequent weeks of elastomeric chain use, and there were no significant main effects among any of the independent variables. The estimated marginal means and associated confidence intervals for the EL and SL across the two time points are presented in Table IV (p. 46).

Findings of ATP-Driven Bioluminescence: The raw mean ATP-driven bioluminescence values of the EL and SL brackets are shown in Table II (p. 44). The results from the full factorial ANOVA are presented in Table V (p. 47). There was a significant three-way interaction among bracket type, arch, and time, $F(1,8)=7.308$, $p=.027$, partial $\eta^2=.477$. The estimated marginal means and associated confidence intervals are presented in Table VI (p.48). The bracket time by type interaction for the maxillary arch is presented in Figure 8, Panel A and for the mandibular arch in Figure 8, Panel B (p.54). ATP-driven bioluminescence values collected from teeth with EL and SL brackets in the mandibular arch appear to exhibit similar change following introduction of elastomeric chain use, from one year (EL Geometric mean (GM)= 1.499E+08; SL GM= 1.271E+08) to one year and five weeks later (EL GM= 1.525E+08;SL GM= 1.089E+08). However, there was a differential change in ATP-driven bioluminescence values following five weeks of elastomeric chain use for the EL and SL brackets in the maxillary arch. For the maxillary arch, ATP-driven bioluminescence values for the teeth with EL (GM= 8.192E+07) and SL (GM= 6.950E+07) brackets were similar at one

year, but the ATP-driven bioluminescence values appeared to increase for the teeth fitted with SL brackets after five further weeks of elastomeric chain (GM=1.298E+08) and remained the same for teeth fitted with EL brackets (GM= 6.726E+07).

Correlations Between Bacterial Number and ATP-Driven Bioluminescence Values: In comparisons between total bacterial numbers vs. ATP-driven bioluminescence values, using the entire data set for all plaque and saliva specimens, we determined that ATP-driven bioluminescence correlated strongly with total oral bacterial numbers at one year post-bonding ($r= 0.91$, $p<0.05$) and after five additional weeks of elastomeric chain ($r=0.78$, $p<0.05$). When analyzing data from both collection times in one data set, ATP-driven bioluminescence values strongly correlated to the numbers of total oral bacteria ($r=0.85$, $p<0.05$).

WSL Findings: The modified Gorelick index scores for photographic evaluation showed WSL were present on 7 of the 50 (14%) lateral incisors (Table VII; p. 49). One subject had slight visual WSL formations (score of 1) on all four lateral incisors and two other subjects had slight visual WSL present on one or two of the lateral incisors. For the DIAGNOdent readings, 11 of the 50 (22%) of the lateral incisors had a reading ≥ 5 ("lesion"). Seven teeth had readings of 5-10 ("slight lesion"), and three had readings ≥ 11 ("severe lesion"). DIAGNOdent method error was determined to be 0.29 units. The DIAGNOdent positively identified five of the teeth that scored a visual WSL with a reading ≥ 5, however, there were six teeth with no visual WSL that had DIAGNOdent measurements ≥ 5. Two teeth with slight visual WSL had DIAGNOdent measurements < 4 (Table VII; p. 49). The sensitivity and specificity of the DIAGNOdent WSL measurements was computed to be 0.45 and 0.82, respectively, when compared to the modified Gorelick WSL index scores.

DISCUSSION

In this longitudinal 1-year follow-up study to that of Pellegrini and colleagues (2009), two brackets types were utilized to assess the accumulation of plaque bacteria associated with different ligation mechanisms. Of the 14 subjects who completed the original Pellegrini et al. (2009) study, 13 were available for the 1-year specimen collection, and 12 patients completed follow-up after the 5 weeks of elastomeric chain. In the Pellegrini et al. (2009) study, higher total bacterial numbers were obtained surrounding the EL brackets than the SL brackets at one week post-bonding ($p=0.017$) and 5 weeks post-bonding ($p=0.032$; Table II; p. 44). Forsberg et al. (1991) found similar results to Pellegrini et al. (2009), showing that ligation with elastomeric rings was associated with higher mean numbers of bacteria at every collection time point compared to ligation with steel wires. The Forsberg et al. (1991) study included 12 subjects where on one side of the midline brackets were ligated with elastomeric rings and the other side with steel ligatures. Their plaque samples were collected at 4, 10, 19, 34, and 61 weeks using charcoaled points on the labial surface of upper lateral incisors around the circumference of the brackets (Forsberg et al. 1991). The method of ligation was switched at each collection time with the opposite side of the midline such that the side that was previously tied with elastomeric rings was interchanged to the side tied with steel ligatures. For the Forsberg et al. (1991) study, bacterial cultivation analyses were also performed using plaque and saliva samples to determine total bacteria, *S. mutans* and lactobacilli numbers.

Contrary to our earlier findings in the Pellegrini et al. (2009) study and the implications of the Forsberg et al. (1991) study , it was determined in the current study that with different orthodontic brackets the significant difference in plaque bacterial load found early in orthodontic treatment disappears after one year of orthodontic treatment. Pellegrini et al. (2009) found

higher numbers of total bacteria surrounding EL brackets compared to SL brackets (Table II; p. 44). Pellegrini et al. (2009) also observed a trend toward equivalency between the amounts of total bacteria surrounding the two different brackets from one week to five weeks post-bonding. The differences in total plaque bacteria surrounding the two bracket types found early at one week post-bonding, and then disappearing by one 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.

The observations of Turkkahraman and associates (2005) 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 upper 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, one week after bonding, and at five weeks (Turkahraman et al. 2005). Turkkahraman et al. (2005) found that, although microbial counts were slightly numerically higher in the elastomeric group, there was no significant difference between groups. No previous studies have evaluated the difference in plaque bacterial load surrounding EL vs. SL brackets with the use of elastomeric chain. It was found that when elastomeric chains were introduced, and as assessed using ATP-driven bioluminescence determinations, SL brackets in the upper arch were associated with more plaque bacteria than were EL brackets (Figure 8, Panels A and B; p. 54). It may be that the introduction of elastomeric chain, along with the ligating gate of SL brackets, may prevent removal of plaque due to the ligating gate precluding plaque removal from within

the lumen of the chain links. This result suggests that oral hygiene instructions should be reinforced at appointments when elastomeric chain is placed.

It is interesting to note that the mean total plaque bacteria decreased when elastomeric chain was introduced for five further weeks compared to one year post-bonding (Table II; p. 44), and although these differences were not statistically-significant, we anticipated that elastomeric chain use would result in increases in total plaque bacteria. It is possible that patients had increased awareness from the plaque collection that occurred at the one year sampling appointment, with this instituting an improvement in oral hygiene practices during the five further weeks of elastomeric chain use prior to the next sampling appointment. We were unable to find evidence in the literature that documents an increase in plaque bacteria associated with the use of elastomeric chain compared to individual elastic modules, however it is a common clinical observation that elastic chains are major plaque traps.

In this study ATP-driven bioluminescence was studied to assess whether it may be used as a rapid tool for the quantification of total oral bacteria. As with the results of Pellegrini et al. (2009) where ATP-driven bioluminescence values correlated well with total oral bacterial numbers ($r= 0.90$), in this study ATP-driven bioluminescence was also found to correlate strongly with total oral bacterial numbers at one year post-bonding ($r= 0.91$) and after five additional weeks of elastic chain ($r=0.78$). 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 chair-side 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 WSL (Pellegrini et al. 2009).

Unlike the findings by Pellegrini et al. (2009) with the same subjects, the current study found a significant interaction of time, bracket type, and arch with respect to ATP-driven bioluminescence values. In the maxillary arch only, bacterial load as assessed by ATP-driven bioluminescence values was found to be retained more predominantly surrounding SL brackets than EL brackets after five weeks of elastomeric chain use, whereas there were no differences between the SL and EL brackets over the five weeks for the mandibular arch.

In the current study, no differences in WSL were found between the different brackets, though only minimal visual-photographic WSL were found. Similarly, in a study by Polat and associates (2008), no significant differences in WSL formation were found between self-ligating and conventional brackets. Polat et al. (2008) evaluated two groups of 10 patients, one group was bonded with In-ovation self-ligating brackets and the other group was bonded with Ovation conventional brackets (the same brackets were evaluated in the current study). WSL were scored similar to our study by using a Gorelick index: 0, no WSL; 1, WSL covering less than one-third; 2, WSL covering more than one-third; 3, WSL with cavitation (Gorelick et al. 1982). WSL formation was found to be no different surrounding the two different brackets as evaluated using the Mann Whitney *U* test (Polat et al. 2008).

In the present study the DIAGNOdent was found to accurately identify only 5 visual-photographic WSL (sensitivity of 0.45). However, the DIAGNOdent was found to more accurately identify absence of visual-photographic WSL (specificity of 0.82). Of the 50 teeth involved in our study, only 3 teeth (6%) had DIAGNOdent measurements above 10, a reading considered to represent significant lesions according to recommendations of the manufacturer. In a study by Gorelick et al. (1982) where orthodontic patients were evaluated visually for WSL, the prevalence of WSL was 10.8% of the 2,211 teeth examined. They found the prevalence of

WSLs among maxillary lateral incisors to be 23% (Gorelick et al. 1982), compared to the current findings of 14% using the visual-photographic evaluation (Gorelick et al. 1982; scores of ≥1) and 22% using DIAGNOdent (readings ≥5; Table VII; p. 49). Although our DIAGNOdent data compares closely to that of Gorelick et al. (1982) for lateral incisors, surprisingly our evaluation of photographs did not agree. The latter finding may relate to differences in sensitivity of direct visual (Gorelick et al. 1982) versus the photographic method used in the current study, or to differences in the study populations. Our results suggest the prevalence of demineralization and incipient WSL generally may be higher than that found by Gorelick et al. (1982) if DIAGNOdent measurements with a threshold reading of 5 were to be used.

Because the number of teeth that developed WSL was relatively low in the current study, and thus our ability to test for detection of WSL was limited, findings of other investigators who have utilized laser light fluorescence for detecting WSL are of interest. In an *in vitro* study by Staudt and associates (2004), DIAGNOdent measurements were made around orthodontic brackets bonded to extracted third molars. Teeth selected in their 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 (Staudt et al. 2004). 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* (Staudt et al. 2004). 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 (Barberia et al. 2008, Pinelli et al. 2002). Although the specificity (0.82) was found to be reasonably good in the present study, sensitivity was found to be rather low (0.45). Similar to our findings, Kronenberg and associates (2009) recently evaluated the development of WSL *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 (Kronenberg et al 2009). Results of the current study suggest that a larger sample size will be required in order to more fully investigate correlations between WSL formation surrounding EL vs. SL brackets, and between visual evaluations versus DIAGNOdent readings.

CONCLUSIONS

1. Even though early in orthodontic treatment (1-5 weeks post-bonding), SL appliances were found to promote reduced retention of plaque bacteria compared to EL appliances, by one year post-bonding, no differences were found between SL and EL brackets.

2. After 5 weeks of elastomeric chain use, SL appliances in the upper arch were found to promote increased retention of total plaque bacteria compared to EL appliances.

3. ATP-driven bioluminescence values correlated significantly to the total oral bacterial numbers ($r=0.85$, $p<0.05$). ATP-driven bioluminescence may serve as a rapid tool for the quantification of bacterial load used at chair-side during orthodontic treatment.

4. Although the sensitivity of DIAGNOdent for WSL formation was found to be low (0.45) when compared to visual-photographic WSL, further studies should be performed, as the digital DIAGNOdent readout may prove to be a useful, rapid tool for chair-side detection of early WSL formation and patient education.

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TABLE I. Patient Demographics and Placement of Brackets on Lateral Incisors (modified from Pellegrini et al. 2009)

Patient #1	Gender	Age	Handedness	Oral Health	Average Time Brushing Before Visits (hrs)2	Orthodontic Treatment3	(UR) Tooth 7 Bracket Type4	(UL) Tooth 10 Bracket Type4	(LL) Tooth 23 Bracket Type4	(LR) Tooth 26 Bracket Type4
1	M	14.6	Right	good	1	full	EL	SL	EL	SL
2	M	14.11	Right	good	2	full	EL	SL	EL	SL
3	F	16.1	Right	good	1	full	SL	EL	SL	EL
4	F	14	Right	good	1	full	SL	EL	SL	EL
5	F	17.2	Ambidextrous	good	1	upper	EL	SL	N/A	N/A
6	F	12.2	Right	good	1	full	SL	EL	SL	EL
7	F	12.9	Right	good	4	full	EL	SL	EL	SL
8	F	15.9	Right	good	5	full	EL	SL	EL	SL
12	F	12.11	Left	good	3	full	EL	SL	EL	SL
14	F	12.1	Right	good	4	full	SL	EL	SL	EL
16	M	13.11	Right	good	3	full	SL	EL	SL	EL
17	F	15.7	Left	good	3	full	SL	EL	SL	EL
18	M	13.11	Right	fair	5	full	SL	E	SL	E

[1]All Patients live in Portland, OR and surrounding areas

[2]All Patients refrained from eating for at least one hour prior to visits

[3]No patients had active cares when orthodontic appliances were placed

[4]EL = elastomeric-ligating bracket; SL = self-ligating bracket

TABLE II. Composite from the Present Study and Pellegrini et al. (2009) of Total Bacterial Numbers and ATP-driven Bioluminescence Units on Teeth Ligated with Elastomeric-Ligating and Self-Ligating Brackets

	Self-Ligating		Elastomeric-Ligating		Self-Ligating		Elastomeric-Ligating	
	Total Bacteria				ATP			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
One Week Post-Bonding†	2.00E+06	2.46E+06	5.00E+06	7.59E+06	3.56E+07	3.65E+07	7.80E+07	9.02E+07
Five Weeks Post-Bonding†	2.00E+06	4.23E+06	3.00E+06	4.68E+06	8.90E+07	1.21E+08	1.18E+08	1.31E+08
One Year Post-Bonding	1.52E+07	2.11E+07	1.13E+07	1.66E+07	2.20E+08	4.09E+08	2.21E+08	3.61E+08
One Year Plus Five Weeks Post-Bonding	7.73E+06	1.25E+07	6.38E+06	1.42E+07	1.52E+08	1.13E+08	1.19E+08	7.87E+07

† Time points containing data collected in the Pellegrini et al. 2009 study

Table III. Analysis of Variance for Log of Total Bacterial Numbers

Source	df	F	P	Partial η2
Pattern of bracket assignment	1	.168	.693	.021
Error	8			
Arch	1	1.235	.299	.134
Arch * Pattern of bracket assignment	1	.025	.878	.003
Error(Arch)	8			
Bracket type	1	.371	.560	.044
Bracket type * Pattern of bracket assignment	1	.512	.495	.060
Error(Bracket type)	8			
Time	1	.443	.524	.053
Time * Pattern of bracket assignment	1	1.274	.292	.137
Error(Time)	8			
Arch * Bracket type	1	.239	.638	.029
Arch * Bracket type * Pattern of bracket assignment	1	.757	.410	.086
Error(Arch*Bracket type)	8			
Arch * Time	1	.086	.776	.011
Arch * Time * Pattern of bracket assignment	1	.357	.567	.043
Error(Arch*Time)	8			
Bracket type * Time	1	1.193	.306	.130
Bracket type * Time * Pattern of bracket assignment	1	1.551	.248	.162
Error(Bracket type*Time)	8			
Arch * Bracket type * Time	1	.074	.793	.009
Arch * Bracket type * Time * Pattern of bracket assignment	1	.072	.795	.009
Error(Arch*Bracket type*Time)	8			

Note: df = degrees of freedom, F = F ratio, p = Type I error probability of given F ratio, partial η2 = proportion of variance accounted for in dependent variable by a factor, excluding the variance from other factors

TABLE IV. Natural Log Transformed and Back Transformed Total Bacterial Numbers on Teeth Ligated with Elastomeric-Ligating and Self-Ligating Brackets

		Transformed				Back Transformed (Geometric Means)		
				95% Confidence Interval			95% Confidence Interval	
Time	Type	Mean	Std. Error	Lower Bound	Upper Bound	Mean	Lower Bound	Upper Bound
One Year	SL	15.072	.585	13.723	16.420	3.512E+06	9.121E+05	1.352E+07
	EL	15.224	.556	13.942	16.506	4.089E+06	1.135E+06	1.473E+07
One Year + Five weeks	SL	14.955	.396	14.041	15.868	3.125E+06	1.253E+06	7.791E+06
	EL	14.522	.407	13.582	15.461	2.026E+06	7.919E+05	5.184E+06

Table V. Analysis of Variance for Log of ATP-driven Bioluminescence

Source	df	F	P	Partial η2
Pattern of bracket assignment	1	.142	.717	.017
Error	8			
Arch	1	17.445	.003	0.686*
Arch * Pattern of bracket assignment	1	1.634	.237	.170
Error(Arch)	8			
Bracket type	1	.000	.985	.000
Bracket type * Pattern of bracket assignment	1	2.543	.149	.241
Error(Bracket type)	8			
Time	1	.072	.795	.009
Time * Pattern of bracket assignment	1	.206	.662	.025
Error(Time)	8			
Arch * Bracket type	1	.593	.463	.069
Arch * Bracket type * Pattern of bracket assignment	1	.946	.359	.106
Error(Arch*Bracket type)	8			
Arch * Time	1	1.365	.276	.146
Arch * Time * Pattern of bracket assignment	1	1.282	.290	.138
Error(Arch*Time)	8			
Bracket type * Time	1	2.267	.171	.221
Bracket type * Time * Pattern of bracket assignment	1	.492	.503	.058
Error(Bracket type*Time)	8			
Arch * Bracket type * Time	1	7.308	.027	0.477*
Arch * Bracket type * Time * Pattern of bracket assignment	1	4.373	.070	.353
Error(Arch*Bracket type*Time)	8			

Note: df = degrees of freedom, F = F ratio, p = Type I error probability of given F ratio, partial η2 = proportion of variance accounted for in dependent variable by a factor, excluding the variance from other factors

* = $p<.05$

TABLE VI. Natural Log Transformed and Back Transformed ATP-driven Bioluminescence Units on Teeth Ligated with Elastomeric-Ligating and Self-Ligating Brackets

			Transformed				Back Transformed (Geometric Means)		
					95% Confidence Interval			95% Confidence Interval	
Arch	Type	Time	Mean	Std. Error	Lower Bound	Upper Bound	Mean	Lower Bound	Upper Bound
Lower	SL	One Year	18.661	.347	17.861	19.460	1.271E+08	5.713E+07	2.828E+08
		One Year + Five Weeks	18.506	.275	17.871	19.141	1.089E+08	5.770E+07	2.054E+08
	EL	One Year	18.826	.207	18.349	19.302	1.499E+08	9.312E+07	2.414E+08
		One Year + Five Weeks	18.843	.174	18.441	19.245	1.525E+08	1.020E+08	2.281E+08
Upper	SL	One Year	18.057	.384	17.172	18.942	6.950E+07	2.869E+07	1.684E+08
		One Year + Five Weeks	18.682	.238	18.132	19.231	1.298E+08	7.495E+07	2.248E+08
	EL	One Year	18.221	.528	17.005	19.438	8.192E+07	2.427E+07	2.765E+08
		One Year + Five Weeks	18.024	.212	17.535	18.513	6.726E+07	4.124E+07	1.097E+08

TABLE VII. Comparison of Visual WSL Indicies with Diagnodent WSL Measurements

	Site 1		Site 2		Site 3		Site 4	
Patient #	DIAGNOdent reading	Visual WSL	DIAGNOdent reading	Visual WSL	DIAGNOdent reading	Visual WSL	DIAGNOdent reading	Visual WSL
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	+	+	+	+	-	-	+	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	n/a	n/a	n/a	n/a
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	+	-
12	+	-	-	-	-	-	-	-
14	-	-	-	-	+	-	+	-
16	+	+	-	-	-	-	+	-
17	-	-	-	-	-	-	-	-
18	+	+	+	+	-	+	-	+

+ indicates a positive finding of WSL using the DIAGNOdent (reading $\geq$5) or visual Modified Gorlick Index (score $\geq$1)

- indicates a negitive finding of WSL using the DIAGNOdent or visual Modified Gorlick Index

For any given patient at one site, identical signs indicate an aggreement between WSL methods

For any given patient at one site, conflicting signs indicate a disaggreement between WSL methods

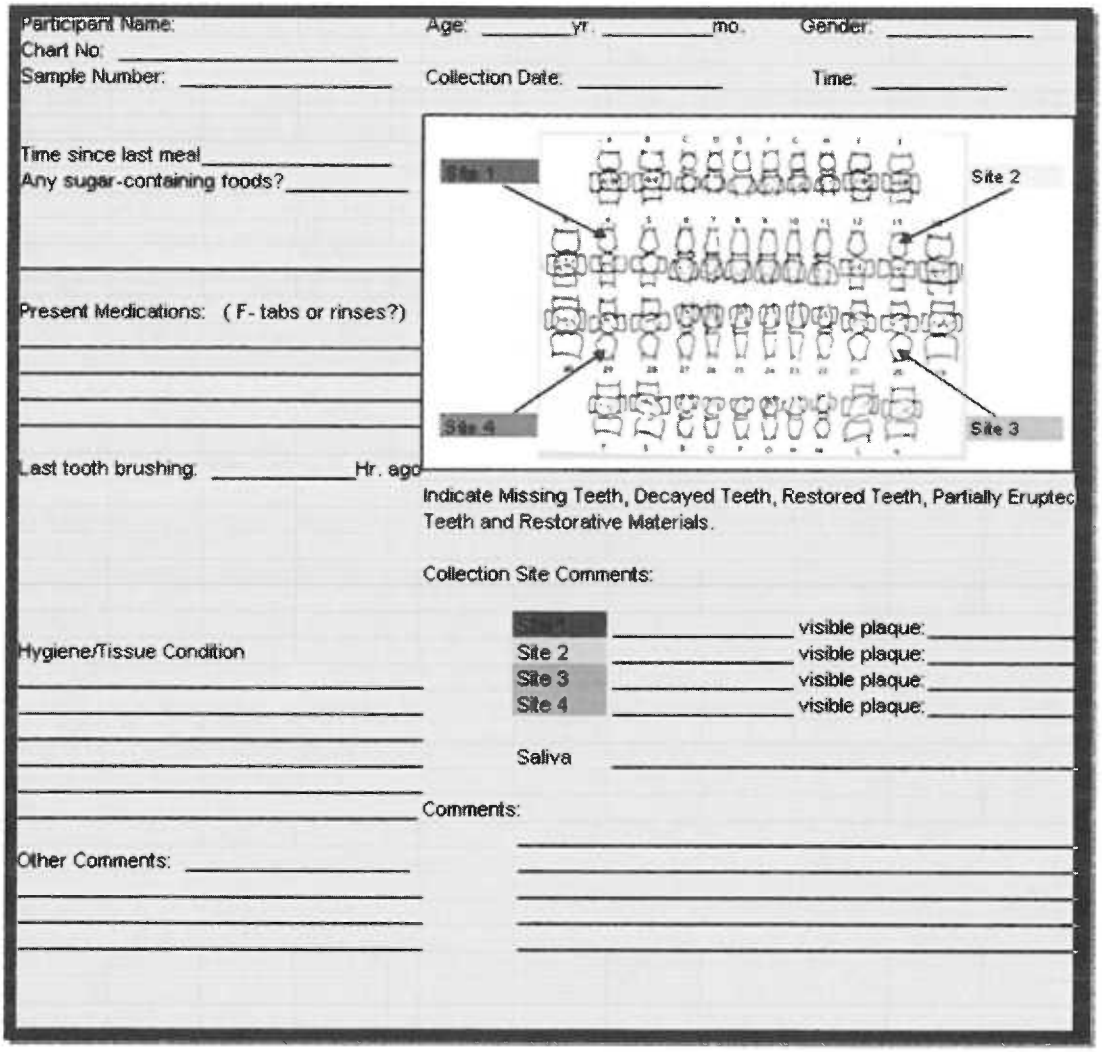

Figure 1. The Data Collection sheet used at each visit.

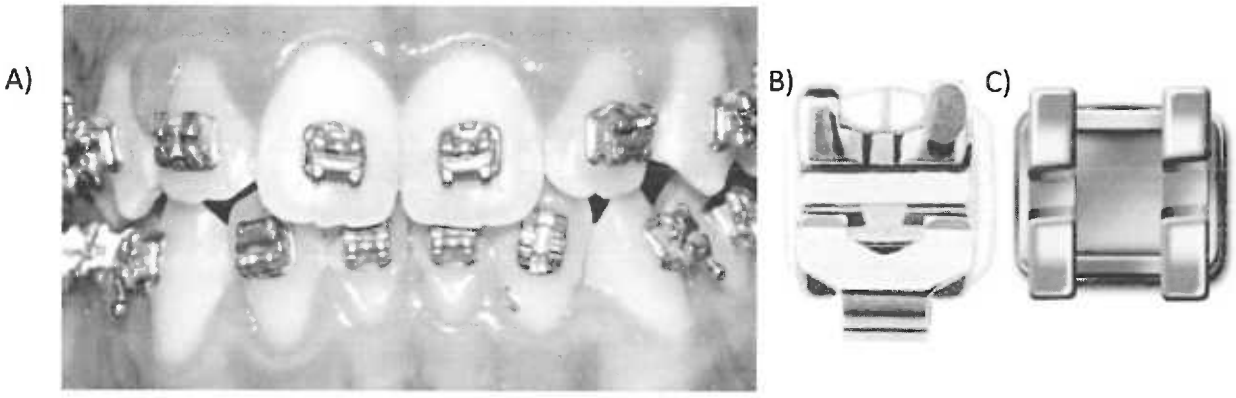

Figure 2. (A) Clinical split mouth design, upper and lower arch, (B) Self-ligating bracket, (C) Elastomeric-ligating bracket.

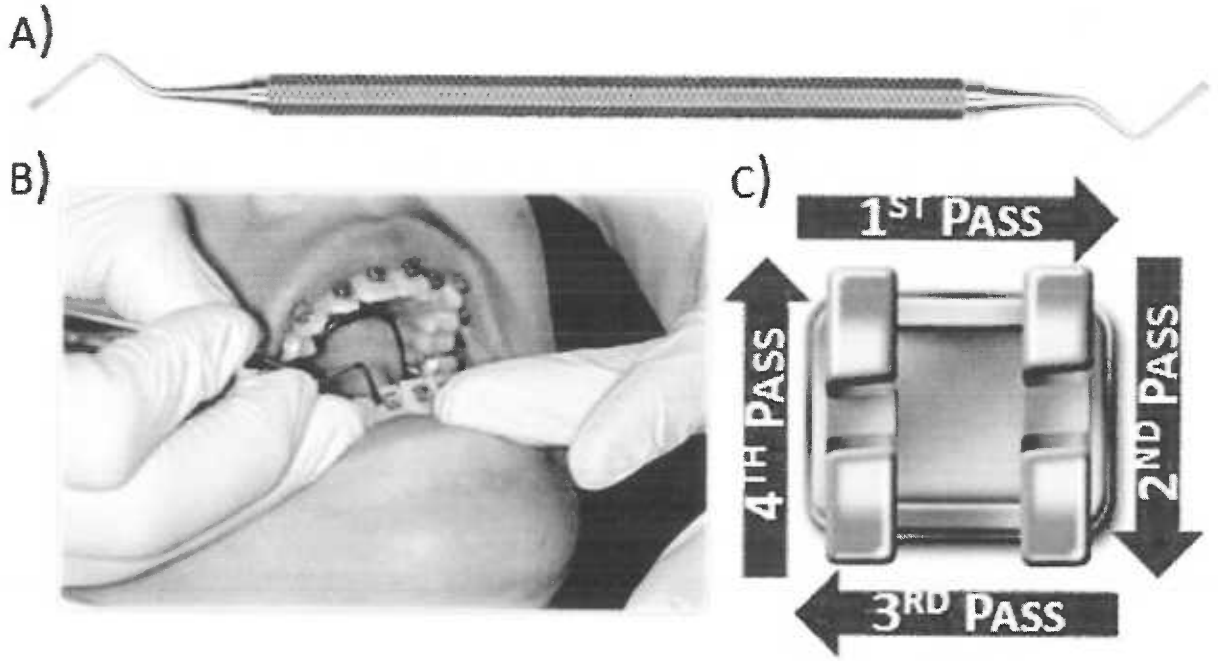

Figure 3. (A) The instrument used for specimen collection: #8/9 Orban® DE hoe scaler (Hu-Friedy, Chicago, Illinois) has a 90°, 1.5 mm tip dimension for standardized and consistent specimen collection, (B) Photo of the specimen collection process, (C) Four-pass sampling technique; the sterilized # 8/9 Orban® DE hoe scaler tip is moved circumferentially around the bracket. The ligation mechanism and archwire were carefully removed prior to sampling.

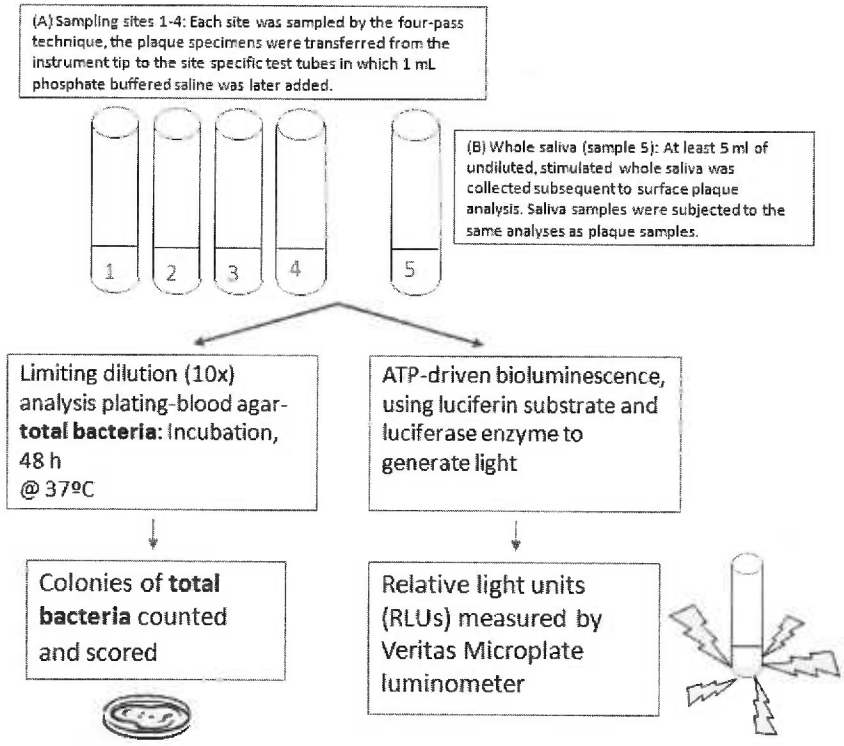

Figure 4. Flow diagram of sample analysis.

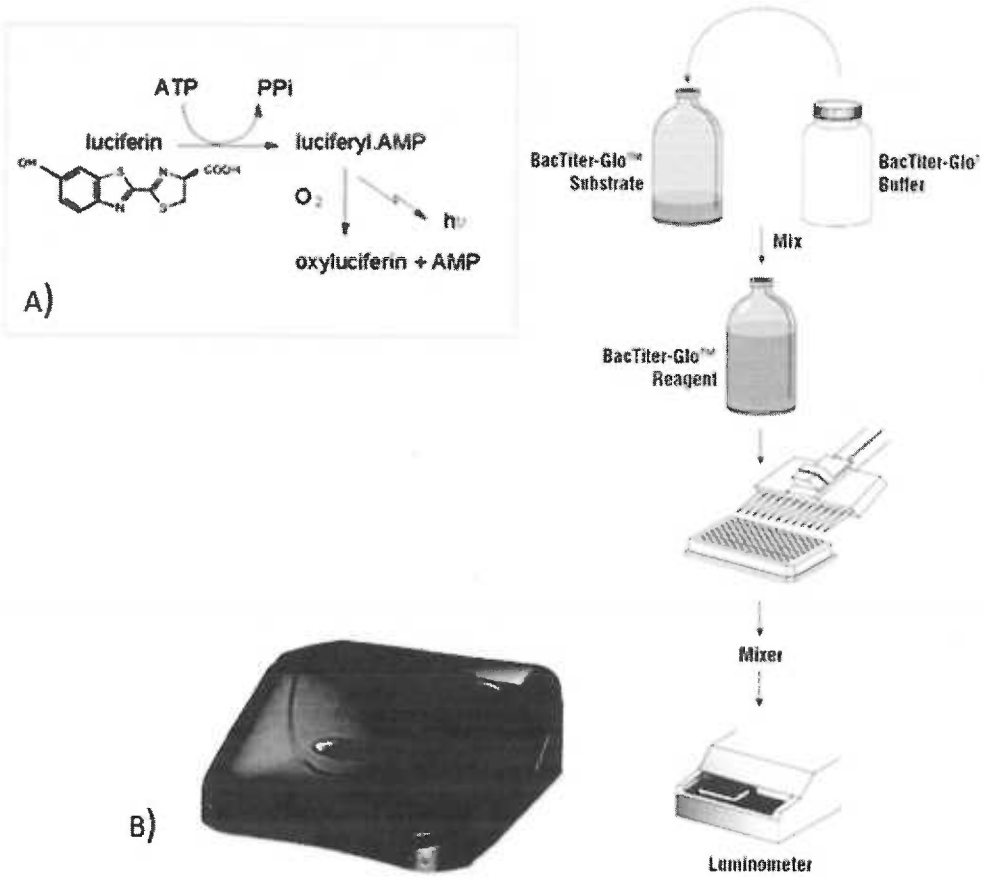

Figure 5. (A) The luciferin-luciferase reaction; (B) The Veritas® Benchtop luminometer, and schematic illustration.

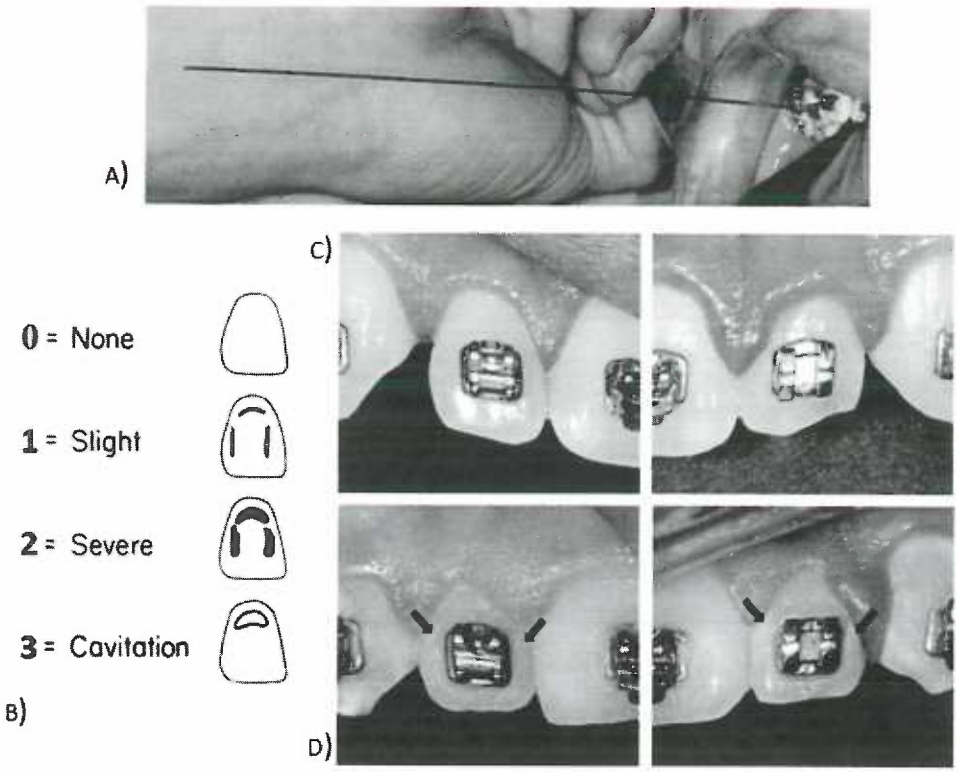

Figure 6. (A) 15cm jig used to standardize photographs (position the camera lens) with black paper as a background, (B) Schematic representation of how lesions were scored (modified from Gorelick et al. 1982), (C AND D) Standardized photographs showing lateral incisors without and with WSL.

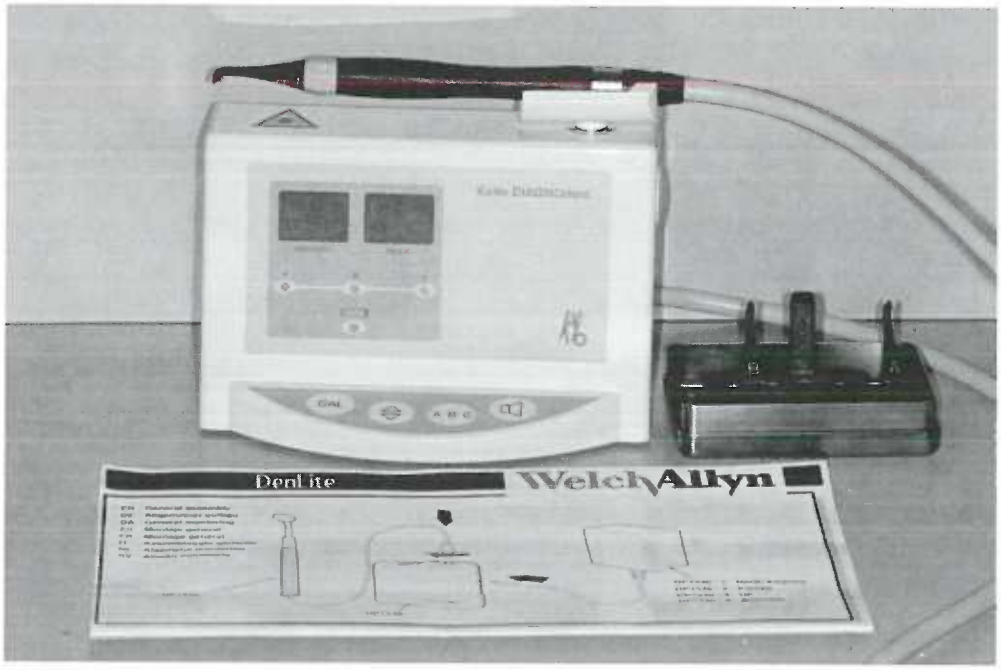

Figure 7. Diagnodent with conical tip (tip A) uses laser-light fluorescence to detect WSL.

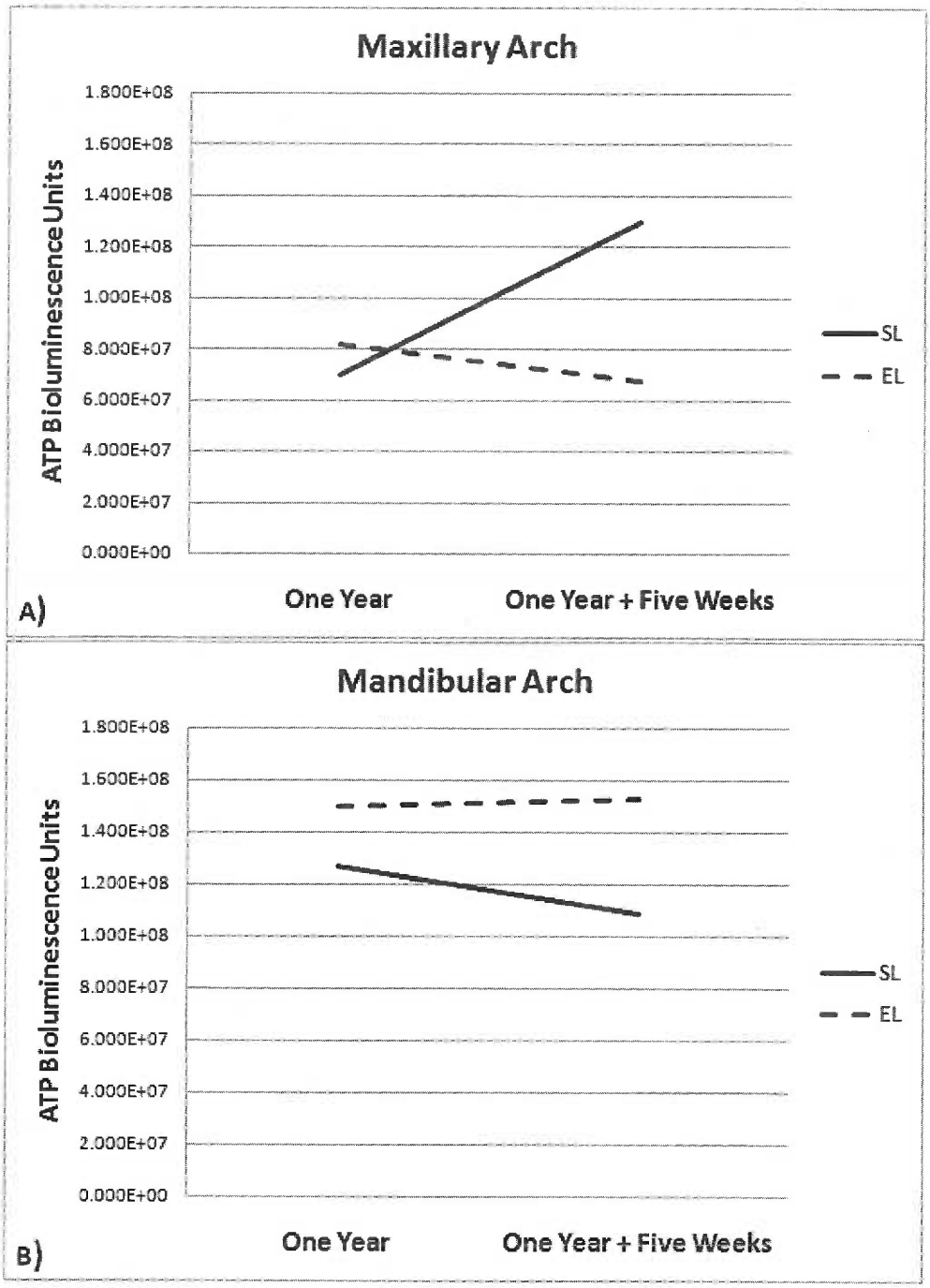

Figure 8. Line plots demonstrating change in ATP-driven bioluminescence values in maxillary and mandibular arches (Panels A and B, respectively), for SL vs. EL brackets from 1-year collection to 1-year plus 5 weeks collection (following use of elastomeric chain). Visual inspection of the pattern of means across time, arch and bracket type are based on the significant F values associated with the three-way interaction involving these variables.

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Self-ligation vs. elastomers: One-year follow-up study examining bracket archwire ligation technique on microbial colonization and white spot lesion formation

ABSTRACT

Objectives: (1) To quantify plaque bacteria at the bracket-tooth interface when using elastomeric-ligating (EL) vs. self-ligating (SL) brackets after one year of orthodontic treatment, (2) to quantify bacteria after elastomeric chain use, (3) to evaluate ATP-driven bioluminescence for quantification of oral bacteria, and (4) to analyze white spot lesions (WSLs) by photographic and laser fluorescence determinations (DIAGNOdent).

Materials and Methods: Thirteen subjects had fixed appliances where lateral incisors were bonded with either EL or SL brackets. Plaque bacteria were collected from incisor surfaces after one year, and after five additional weeks with elastomeric chains, and were quantified using plating methods and ATP-driven bioluminescence. WSLs were evaluated by photographic and DIAGNOdent determinations.

Results: ATP-driven bioluminescence values were similar for teeth with EL (GM= 8.192E+07) or SL (GM= 6.950E+07) brackets at 1 year. After five additional weeks with elastomeric chains, ATP-driven bioluminescence increased for teeth with SL brackets in the maxillary arch only (GM=1.298E+08), with no change observed for teeth with EL brackets. ATP-driven bioluminescence correlated to total bacterial numbers ($r=0.85$, $p<0.05$). DIAGNOdent measurements were found to have low sensitivity (0.45) and good specificity (0.82) when compared to WSL determined using photographic evaluation.

Conclusions: After one year, there were no differences in retention of plaque bacteria or WSL between the two brackets. Following use with elastomeric chains, in the upper arch, SL brackets were associated with increased retention of plaque bacteria. DIAGNOdent may be of limited utility when evaluating WSL surrounding brackets.

Keywords: Elastomeric and self-ligating appliances, plaque retention, white spot lesions

INTRODUCTION

The development of the acid-etch bonding technique has revolutionized the placement of fixed orthodontic appliances. Although bonding of brackets provides many benefits, formation of white spot lesions (WSLs) is a common undesirable side effect. Due to difficulty in cleaning around orthodontic appliances, there is an increase in caries-causing bacteria and plaque accumulation and in development of WSL[1-8]. Cariogenic bacteria, including *Streptococcus mutans*, *Actinomyces* and *Lactobacillus* species, can cause enamel demineralization via accumulation of acid[2,4,5,9]. Metal brackets have been found to enhance the presence of *S. mutans*, which are considered among the first to colonize near brackets[1,2].

There are many variations of fixed orthodontic appliances, but ligation methods are divided into two major categories: conventional elastomeric or wire ligation, and self-ligation. Several studies have evaluated the microbial colonization around conventional brackets associated with ligation wires versus elastomeric modules, with some finding no significant difference in plaque accumulation[5] and others finding increased plaque accumulation with use of elastomeric-ligation[3,10]. Recently, our group[10] quantified plaque bacteria around self-ligating versus conventional elastic-ligating brackets and found significantly lower plaque accumulation around self-ligating brackets at five weeks post-bonding. With reduction in plaque, self-ligating brackets may also promote reductions in WSL development.

Methods for documenting demineralization and WSL formation include digital photography and laser-light fluorescence. Digital photographs are an inexpensive method of evaluating changes in appearance of tooth surface enamel over time. Photographs made from multiple angles can be used to evaluate facial surfaces. In addition, images can be evaluated in random sequence, allowing for assessor blinding and an unbiased detection of WSL[11]. Although

useful for evaluating advanced demineralization, significant mineral loss in enamel is required before WSLs are readily detectable to the eye[8]. Early demineralization can be detected by use of laser-light fluorescence such as the DIAGNOdent, a portable red laser-light fluorescent detector. DIAGNOdent has not been readily used in subjects undergoing fixed orthodontic treatment[7-9,12].

Using the same patient sample set described in Pellegrini et al.[10], we have now conducted a 1-year follow-up study. The objectives of this longitudinal study were to: 1) measure levels of total plaque bacteria at the bracket-tooth interface of EL vs. SL brackets after one year of treatment, 2) compare levels of plaque bacteria between the two bracket types after five additional weeks of elastomeric chain use, 3) evaluate the use of ATP-driven bioluminescence to quantify total oral bacteria, as revalidation of prior determinations described in our earlier Pellegrini et al.[10] study, and 4) to analyze WSL formation on surfaces surrounding both EL vs. SL brackets using visual inspection and laser light fluorescence (DIAGNOdent).

MATERIAL AND METHODS

Patient Demographics, Appliance Placement, and Specimen Collection: The original criteria for selection of subjects in the Pellegrini et al.[10] study were 12 years of age or older at the start of treatment and demonstration of good oral health. Thirteen out of the 14 subjects who finished the Pellegrini et al.[10] study were enrolled in this follow-up study, with one subject (patient #13) dropping out prior to the 1-year follow-up appointment. In addition, patient #8 did not complete the five additional weeks with elastomeric chains. All patients were treated at the 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 using a split-mouth design (Figure 1A). In each arch one lateral incisor received either an "experimental bracket" (self-ligating, 0.022" In-Ovation-R®, GAC International, Bohemia, NY; Figure 1B) or a "control bracket" (standard, elastomeric-ligating, 0.022" Mini-Ovation®, GAC; Figure 1C) and the contralateral incisor received the other type of bracket. Descriptions of the patient population, including bracket-tooth assignments, can be found within Table 1 of Pellegrini et al[10].

Using methods described in Pellegrini et al[10], stimulated saliva was collected and then plaque from all four lateral incisors, with the exception of one patient (#5) where only the upper lateral incisors were included. At the sampling visits, the elastomeric modules were removed or the SL mechanism disengaged and archwires removed. Plaque specimens were obtained around the bracket base of each lateral incisor utilizing a sterilized dental scaler and a four-pass collection technique[10]. Plaque and saliva specimens were collected at an orthodontic adjustment appointment corresponding to one year of treatment. At this appointment, after the archwires were ligated in the SL brackets, elastomeric chains were placed from first molar to first molar in

both arches, with the exception of patient #5. The patients were recalled five weeks later for additional plaque and saliva collections.

Microbiological and ATP-Driven Bioluminescence Determinations: Methods for sample preparation and plating on enriched blood agar, and ATP-driven bioluminescence determinations have been described in Pellegrini et al[10].

Photographs and Laser-Fluorescence Procedures: Photographs were taken immediately after the initial bonding appointment and at the 1-year appointment following plaque collection. Photographic images were obtained using a standardized technique with the placement of a jig (Figure 1D) set at 15 cm distances from the buccal surface of each tooth to the camera lens[11]. Three images of the lateral incisors were made, one perpendicular to the buccal surface and two at approximately 20° above or below perpendicular to the buccal surface as assessed by eye[12]. WSL was scored using a modified Gorelick index[9] with 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 photographs (e.g.: Figure 1E and 1F) were used to correlate visual WSL formation to laser light fluorescence readings.

For laser light fluorescence readings (DIAGNOdent, KaVo America Corp., Lake Zurich, IL), we used techniques similar to those described by Staudt and associates[8]. 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° to the tooth surface to allow measurement of the enamel closest to the bracket[8]. The largest unit (ranging 0-99) was recorded for each of the four sides. Readings from 0-4 correspond to no lesion, 5-10 correspond to slight lesion, and 10-99 correspond to severe lesion. For evaluation of method error, a second set of readings were made on four subjects selected at random. DIAGNOdent method error was

calculated using the Dalberg Formula: $s=\sqrt{\sum d^2/2n}$, 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, and distribution characteristics were calculated. The distributions for total bacterial numbers and ATP-driven bioluminescence determinations were severely positively skewed, and were subjected to natural logarithmic transformation. All statistical analyses were performed on the transformed variables, and means and standard deviations were conducted on the back-transformed variables to bring values back to the original metric.

Two 2 X 2 X 2 X 2 mixed-design full factorial ANOVAs were conducted to determine significant patterns of differences in total bacteria and ATP-driven bioluminescence values between EL and SL brackets. The split-mouth design is an adaptation of the split plot design[13]. This design calls for a within-subjects analysis (repeated measures of teeth and teeth within persons are correlated), which allows for the removal of subject effects from the error term used in ANOVA, and thus increases the power of the analysis. The pattern of bracket assignment was the only between-subjects factor with two levels (EL/SL/EL/SL vs. SL/EL/SL/EL, with the order corresponding to the following teeth: maxillary right lateral incisor, maxillary left lateral incisor, mandibular left lateral incisor, mandibular right lateral incisor). The remainder of the independent variables was within-subjects variables with two levels each: time (1 year vs 1 year plus 5 weeks), bracket assignment (EL vs SL), and arch (maxillary vs mandibular). Significant interactions were plotted to facilitate interpretation.

To explore the correspondence of DIAGNOdent WSL measurements with photographic-visual WSL indices, we calculated the sensitivity (number of correct DIAGNOdent positive WSL identifications/[number of correct DIAGNOdent positive WSL identifications + number of

incorrect DIAGNOdent negative WSL identifications]) and specificity (number of correct DIAGNOdent negative WSL identifications/[number of correct DIAGNOdent negative WSL identifications + number of incorrect DIAGNOdent positive WSL identifications]) of DIAGNOdent measurements.

RESULTS

Plaque Bacterial Load: Raw mean bacterial numbers for total bacteria contained in plaque surrounding the EL and SL brackets are shown in Table 1. Results from full factorial ANOVA of this data are presented in Table 2. There were no significant differences in total plaque bacteria surrounding the two different bracket types at either one year of treatment or after five subsequent weeks of elastomeric chain use, and there were no significant main effects among any of the independent variables. Estimated marginal means and associated confidence intervals for EL and SL across the two time points are presented in Table 3.

ATP-Driven Bioluminescence: Mean ATP-driven bioluminescence values of the EL and SL brackets are shown in Table 1. Results from the full factorial ANOVA of this data are presented in Table 4. There was a significant three-way interaction among bracket type, arch, and time, $F(1,8)=7.308$, $p=.027$, partial $\eta^2=.477$. Estimated marginal means and associated confidence intervals are presented in Table 5. Bracket time by type interaction for the maxillary and mandibular arches are presented in Figure 2A and 2B, respectively. ATP-driven bioluminescence values collected from teeth with EL and SL brackets in the mandibular arch appear to exhibit similar change following introduction of elastomeric chain use, from one year (EL Geometric mean (GM)= 1.499E+08; SL GM= 1.271E+08) to one year and five weeks later (EL GM= 1.525E+08;SL GM= 1.089E+08). However, there was a differential change in ATP-driven bioluminescence values following five weeks of elastomeric chain use for the EL and SL brackets in the maxillary arch. For the maxillary arch, ATP-driven bioluminescence values for teeth with EL (GM= 8.192E+07) and SL (GM= 6.950E+07) brackets were similar at one year, but the ATP-driven bioluminescence values appeared to increase for teeth with SL brackets after

five further weeks of elastomeric chain (GM=1.298E+08) and remained the same for teeth with EL brackets (GM= 6.726E+07).

Correlations Between Bacterial Number and ATP-Driven Bioluminescence Values: In comparisons between total bacterial numbers vs. ATP-driven bioluminescence values, using the entire data set for all plaque and saliva specimens, we determined that ATP-driven bioluminescence correlated strongly with total oral bacterial numbers at one year post-bonding ($r= 0.91$, $p<0.05$) and after five additional weeks of elastomeric chain ($r=0.78$, $p<0.05$). When analyzing data from both collection times in one data set, ATP-driven bioluminescence values strongly correlated to total oral bacterial numbers ($r=0.85$, $p<0.05$).

WSL Findings: Modified Gorelick index scores for photographic evaluation showed WSL were present on 7 of 50 (14%) lateral incisors. One subject had slight visual WSL formations (score of 1) on all four lateral incisors and two other subjects had slight visual WSL present on one or two lateral incisors. For the DIAGNOdent readings, 11 of 50 (22%) of the lateral incisors had a reading ≤ 4 ("no lesion"). Seven teeth had readings of 5-10 ("slight lesion"), and three had readings ≥ 11 ("severe lesion"). DIAGNOdent method error was determined to be 0.29 units. DIAGNOdent positively identified five teeth that scored visual WSLs with readings ≥ 5, however, there were six teeth with no visual WSL and DIAGNOdent measurements ≥ 5. Two teeth with slight visual WSLs had DIAGNOdent measurements < 4. Sensitivity and specificity of the DIAGNOdent WSL measurements were calculated to be 0.45 and 0.82, respectively.

DISCUSSION

In this 1-year follow-up study, two brackets types were utilized to assess accumulation of plaque bacteria associated with different ligation mechanisms. Of the 14 subjects who completed the original Pellegrini et al.[10] study, 13 were available for 1-year specimen collection, and 12 patients completed follow-up after 5 weeks with elastomeric chains. In the Pellegrini et al.[10] study, higher total bacterial numbers were obtained surrounding EL brackets than SL brackets at one and five weeks post-bonding (Table 1). Forsberg et al.[3] 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.

Contrary to our earlier findings in the Pellegrini et al.[10] study and the results of the Forsberg et al.[3] study, the difference in plaque bacterial load found early in orthodontic treatment disappears after one year of orthodontic treatment. The differences in total plaque bacteria surrounding the two bracket types early in orthodontic treatment, and then disappearing by one year, may be due to decreases in patient compliance with oral hygiene practices.

Turkkahraman and associates[5] also found no significant differences in early plaque surrounding brackets ligated by two different methods. Their observations included 21 subjects undergoing orthodontic treatment, in which the upper brackets on the right side were ligated with elastomeric rings and those on the left side were ligated with stainless steel wire ligatures, with microbial samples collected prior to bonding, one week after bonding, and at five weeks[5]. No studies have evaluated differences in plaque bacterial load surrounding EL vs. SL brackets and the use of elastomeric chains. Unlike the findings by Pellegrini et al.[10] with the same subjects, our current study found a significant interaction of time, bracket type, and arch with respect to ATP-driven bioluminescence values. In the maxillary arch only, bacterial load as assessed by

ATP-driven bioluminescence values was found to be retained more predominantly surrounding SL brackets than EL brackets after five weeks of elastomeric chain use, whereas there were no differences between the SL and EL brackets after the five weeks for the mandibular arch (Figure 2A and 2B). The elastomeric chains, along with the ligating gate of the SL brackets, may impede removal of plaque from within the lumen of the chain links.

It is interesting to note that mean total plaque bacteria decreased with five additional weeks of elastomeric chain use (Table 1), and although differences were not statistically-significant, we anticipated that elastomeric chain use would result in increases in total plaque bacteria. Patients may have had an increased awareness from the plaque collection that occurred at the 1-year appointment, with this instituting an improvement in oral hygiene practices.

In this study, ATP-driven bioluminescence was assessed as a rapid tool for quantification of total oral bacteria. As with the results of Pellegrini et al.[10] 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 bacterial numbers at 1-year post-bonding ($r= 0.91$) and after five additional weeks with elastomeric chains ($r=0.78$).

In the present study, the DIAGNOdent was found to accurately identify five visual-photographic WSL (sensitivity of 0.45). However, the DIAGNOdent was found to more accurately identify absence of visual-photographic WSL (specificity of 0.82). Of the 50 teeth examined in our study, only three teeth (6%) had DIAGNOdent measurements above 10, a reading considered to represent significant lesions according to the manufacturer. In Gorelick et al.[6], WSL was identified in 10.8% of the 2,211 teeth examined. Gorelick et al.[6] found prevalence of WSLs among maxillary lateral incisors to be 23% [9], compared to our current findings of 14% using visual-photographic evaluation (Gorelick et al.[6] scores of ≥ 1) and 22% using

DIAGNOdent (readings ≥5). Although our DIAGNOdent data compares closely to that of Gorelick et al.[9] for lateral incisors, surprisingly our evaluation of photographs did not agree. The latter finding may relate to differences in sensitivity of the direct visual method used by Gorelick et al.[6] versus the photographic method used in the current study, or to differences in study populations. Our results suggest that the prevalence of demineralization and incipient WSL may be higher than found by Gorelick et al.[6], if DIAGNOdent measurements with a threshold reading of 5 were used.

CONCLUSIONS

1. Late in orthodontic treatment (1-year post-bonding), no differences in retention of plaque bacteria were found between SL and EL brackets.

2. After five additional weeks of elastomeric chain use, SL appliances in the upper arch were found to promote increased retention of plaque bacteria compared to EL appliances.

3. ATP-driven bioluminescence values correlated significantly to total bacterial numbers ($r=0.85$, $p<0.05$). ATP-driven bioluminescence may serve as a rapid tool for quantification of oral bacterial load that can be used at chair-side during orthodontic treatment.

4. The sensitivity of DIAGNOdent for WSL formation was found to be low (0.45) when compared to visual-photographic WSL. However, further studies are warranted, because of the potential for DIAGNOdent to serve as a rapid tool for chair-side detection of early WSL formation and patient education.

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FIGURE LEGENDS

Figure 1. A) SL and EL brackets bonded on the lateral incisors. B) In-Ovation-R® self-ligating bracket. C) Mini-Ovation® elastic-ligating bracket. D) Stainless steel jig used to standardize the focal distance on the digital camera. E and F) Standardized photographs demonstrating absence or presence of visual WSLs (arrows), respectively.

Figure 2. Line plots demonstrating change in ATP-driven bioluminescence values in maxillary and mandibular arches, for SL vs EL brackets from 1-year collection to 1-year plus 5 weeks collection. Visual inspections of the pattern of means across time, arch and bracket type are based on the significant F values associated with the three-way interaction involving these variables.